Emergent mechanical properties in zebrafish embryonic tissues: theory and experiment

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3/7/2012

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Embryogenesis Explained

Collaborators

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Jared Talbot
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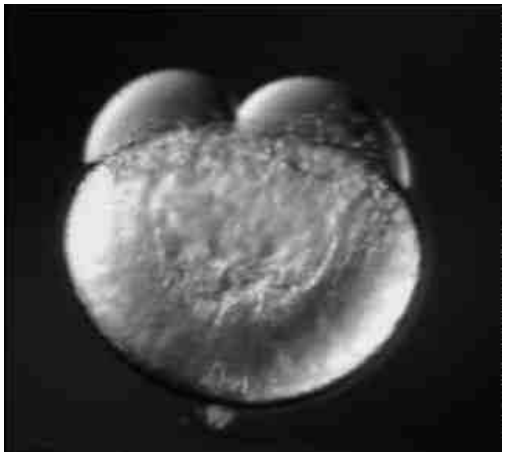
Mal Steinberg




Lewis-Sigler Institute for Genomics, Princeton University

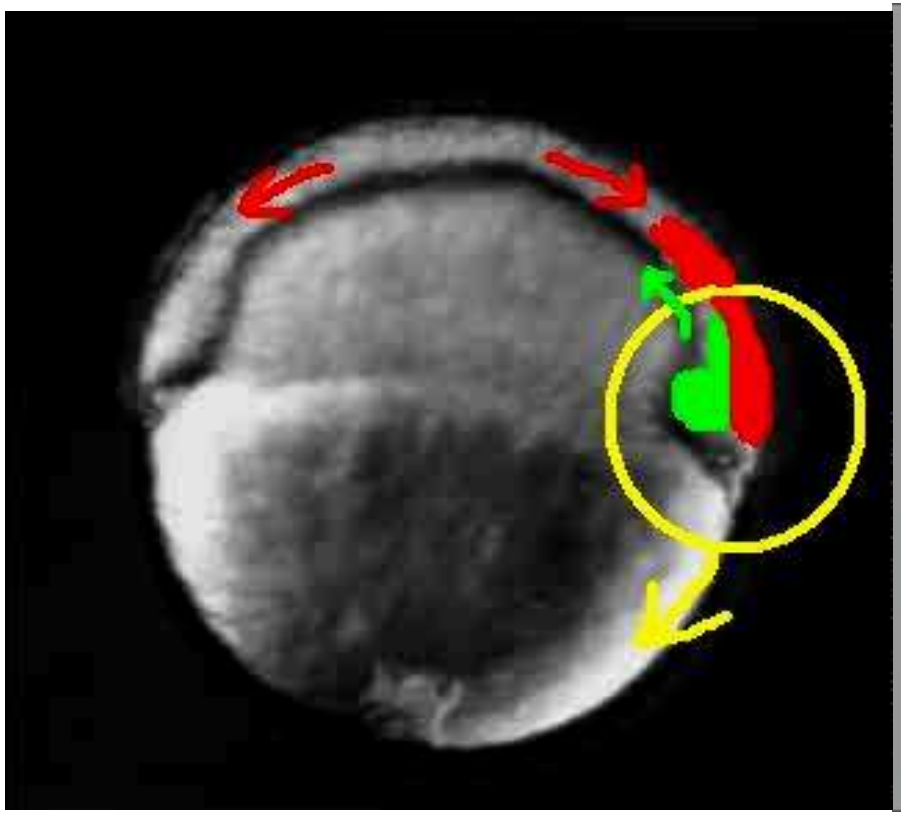
Ramsey Foty
University of Medicine and Dentistry New Jersey

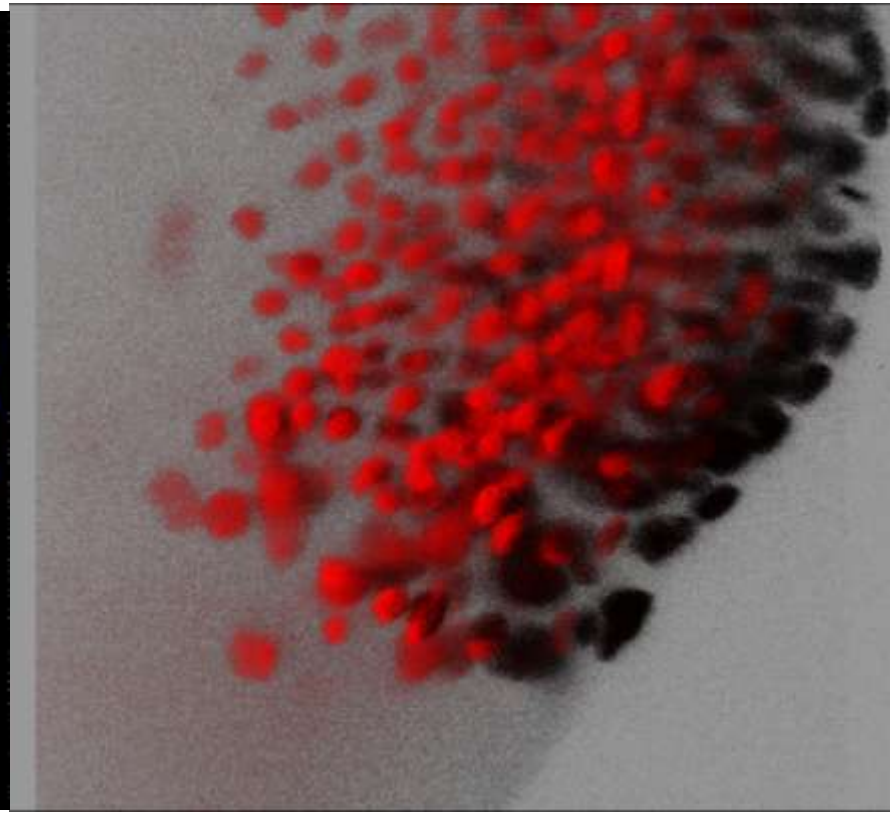
model system: zebrafish aggregates



1. early embryonic development
2. very few cell divisions after initial stage
3. transparent

Goal: Understand cell migration and tissue patterning during early embryonic development





BIG QUESTIONS in embryonic development:

- What role do the mechanical properties of the tissue play in tissue organization during development?
- What properties of single cells give rise to the emergent properties of tissues?
- What are the feedbacks between biochemical signaling and mechanical interactions?

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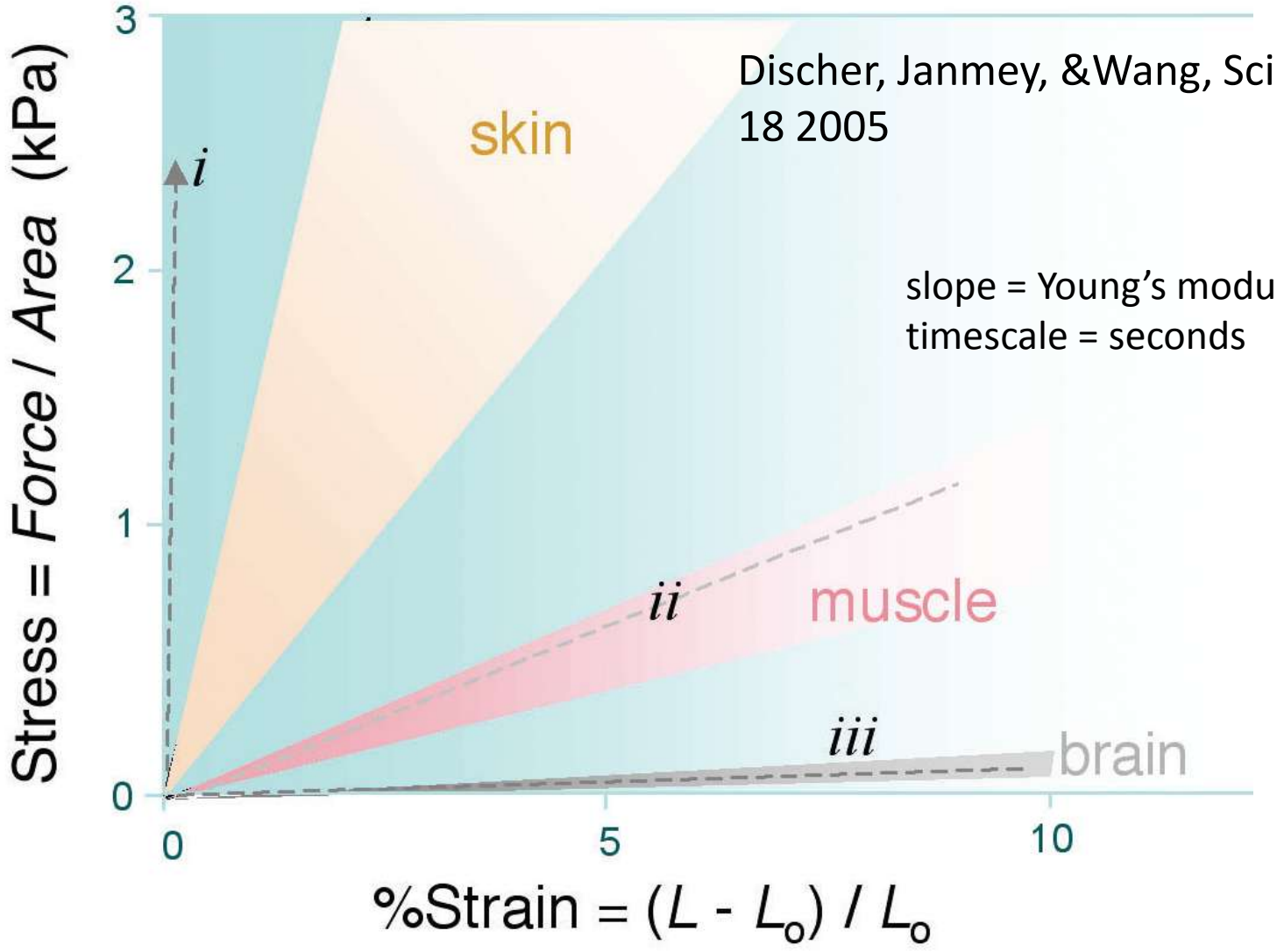
Mechanical properties influence patterning:

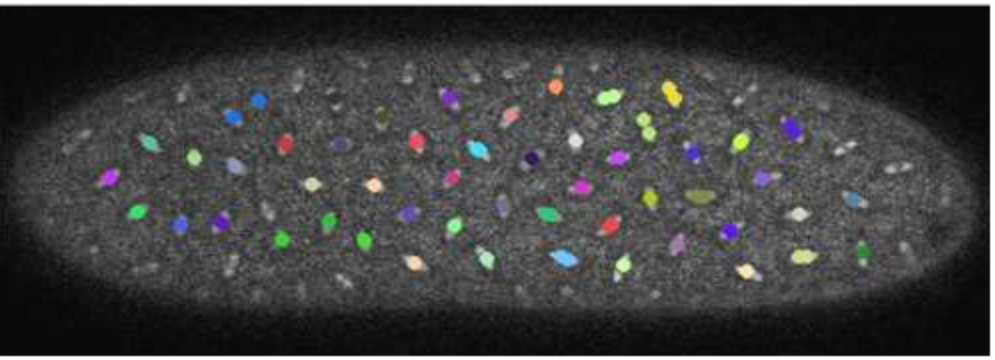


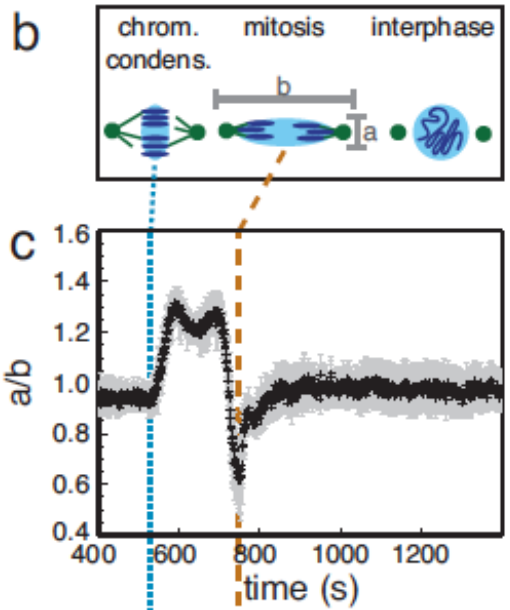
Discher, Janmey, &Wang, Science 310 18 2005

slope = Young's modulus
timescale = seconds

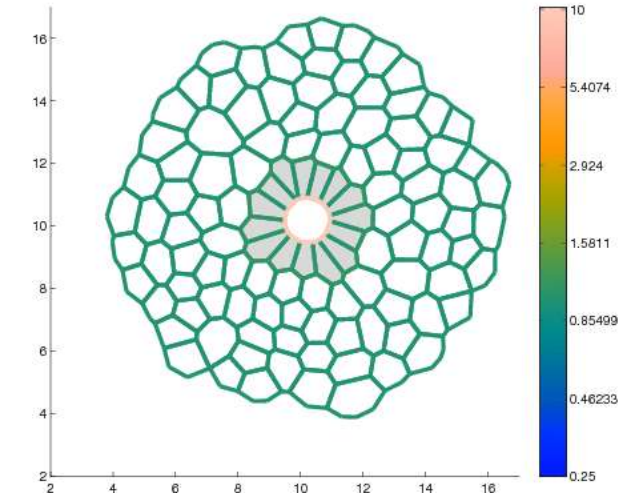
Mechanical properties influence patterning:

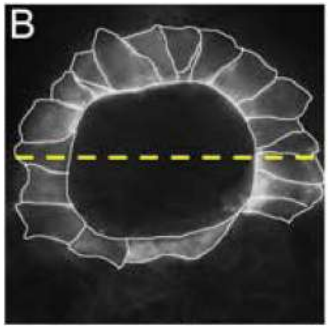
Mitotic waves in drosophila (Idema, Dubuis, MLM, Nelson, Liu, 2012)

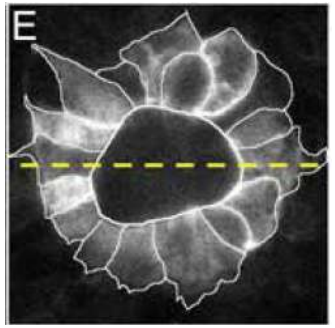




Cell morphology changes in Kupffer's vesicle in zebrafish (Wang, MLM, Amack, 2012)



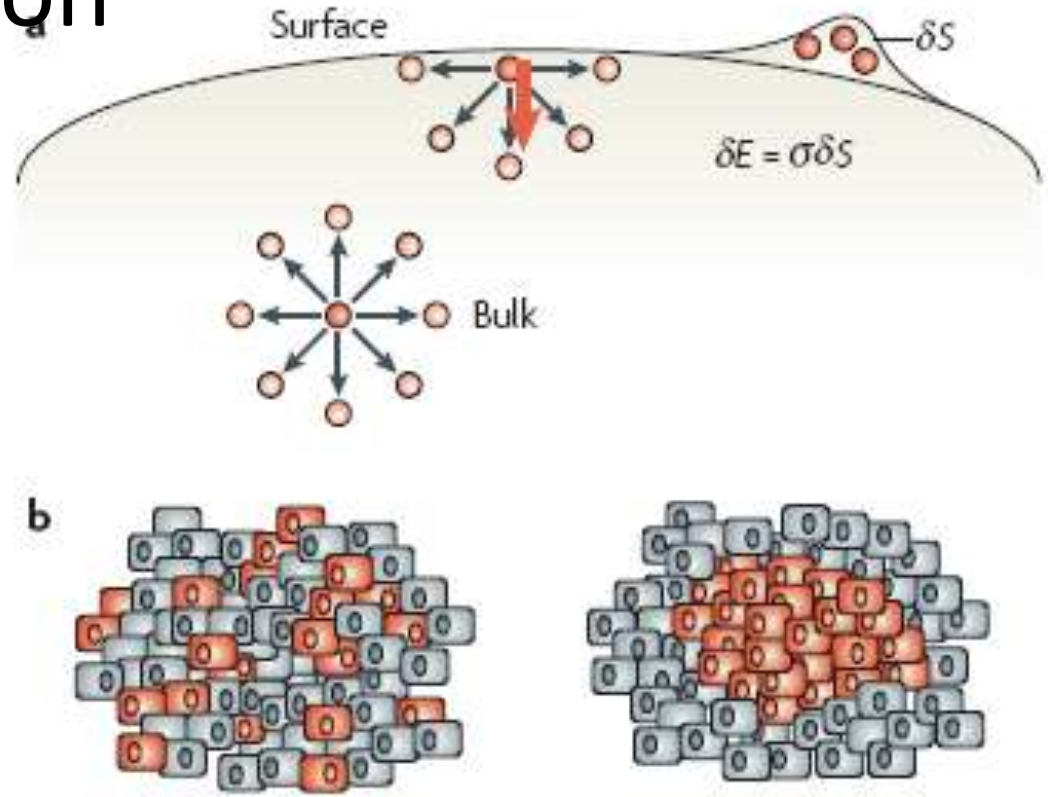




Perhaps most well-known example:

Differential Adhesion Hypothesis (DAH) Steinberg

flow “arise[s] from tissue surface tensions that in turn arise from differences in intercellular adhesiveness”



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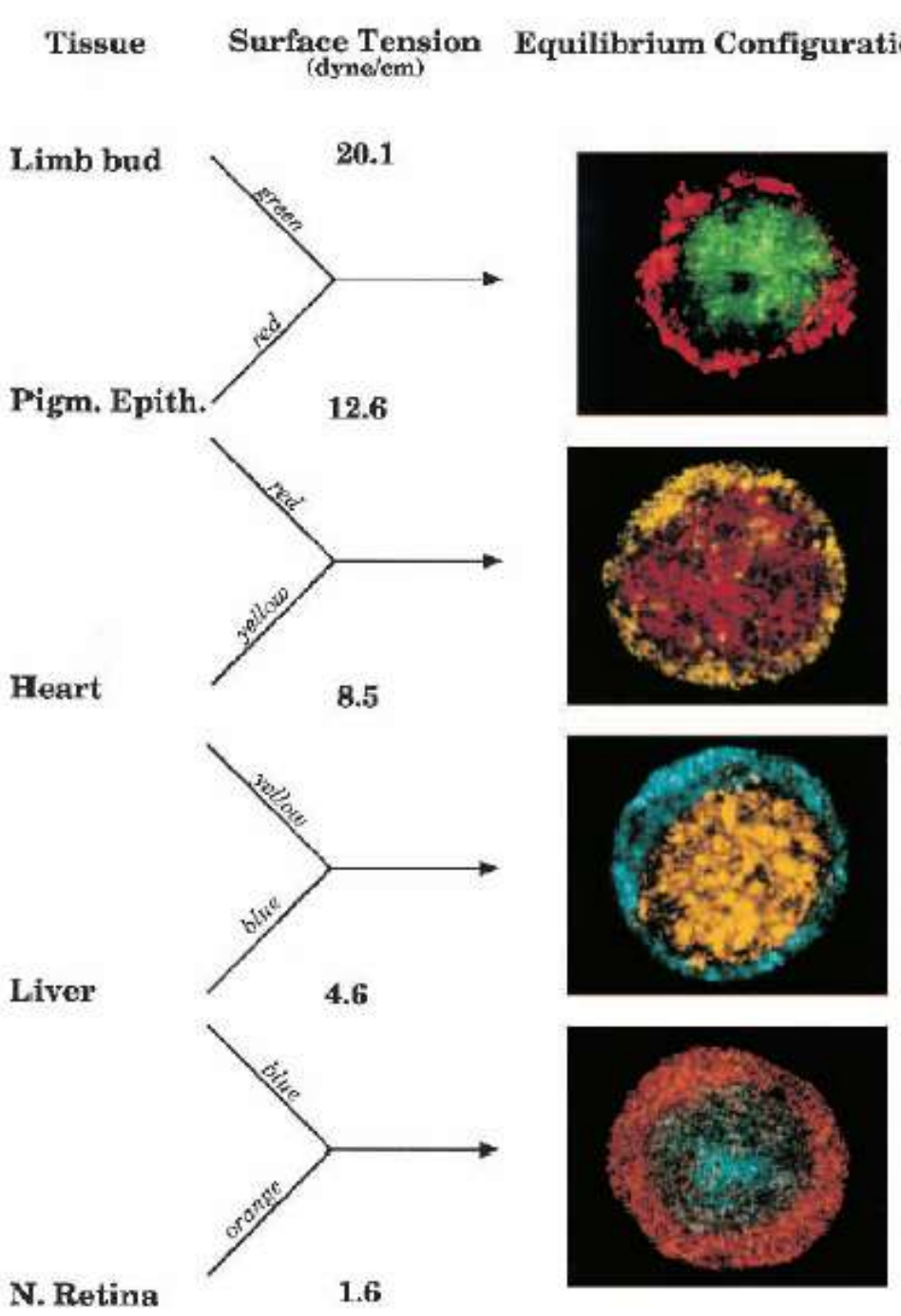
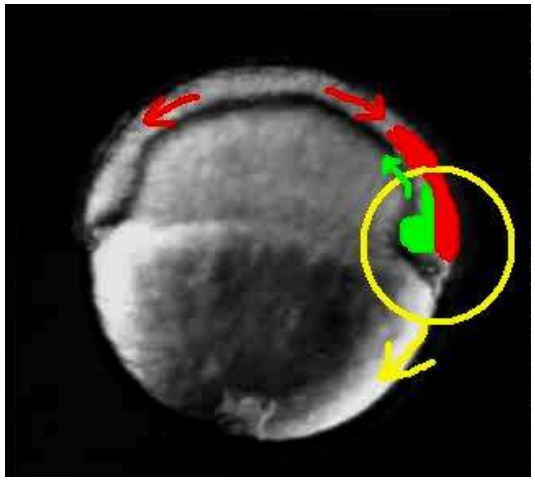
Nature Review, Lecuit and Lenne

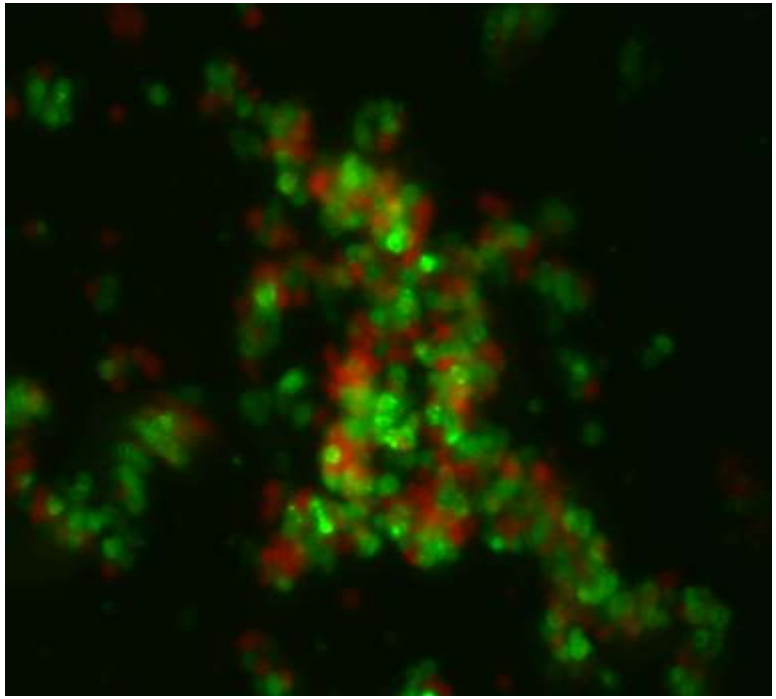


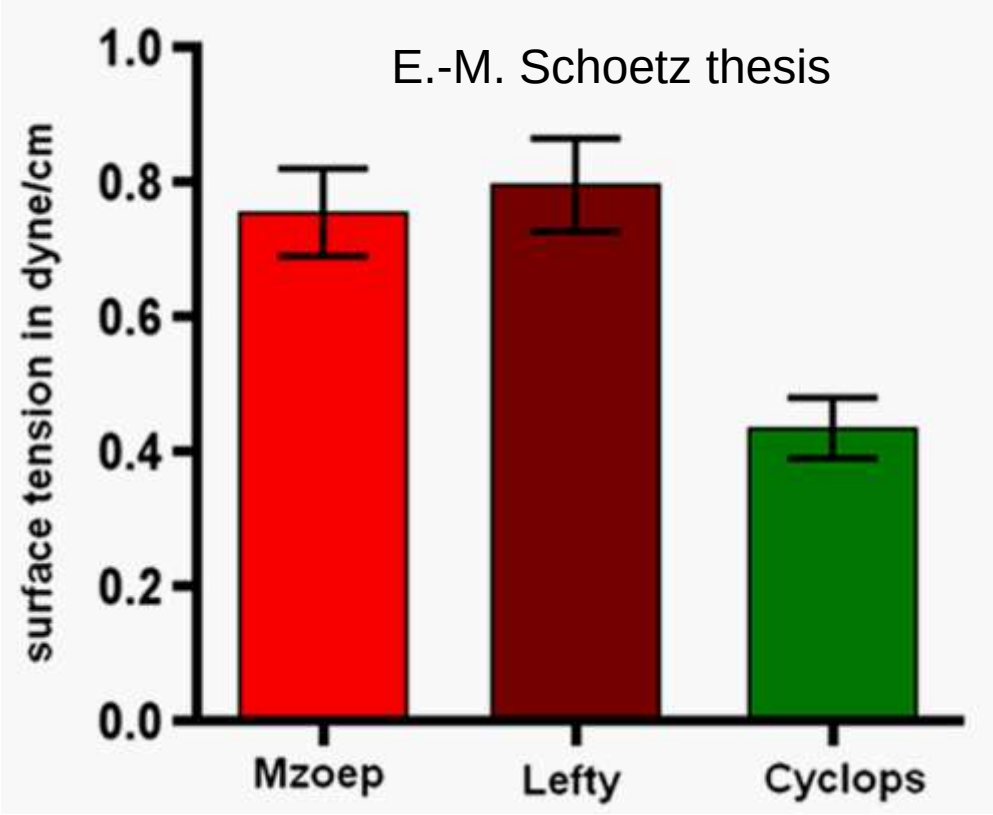
Tissues envelop each other and sort according to their surface tensions

Foty et al,
Development 122, 1611-1620 (1996)

Dynamics for zebrafish embryonic tissue:







tissue	N (total)	σ (dyne/cm)
Mesendoderm	35	0.43 ± 0.04
MZoep (Ecto)	35	0.75 ± 0.06
Lefty (Ecto)	38	0.80 ± 0.07

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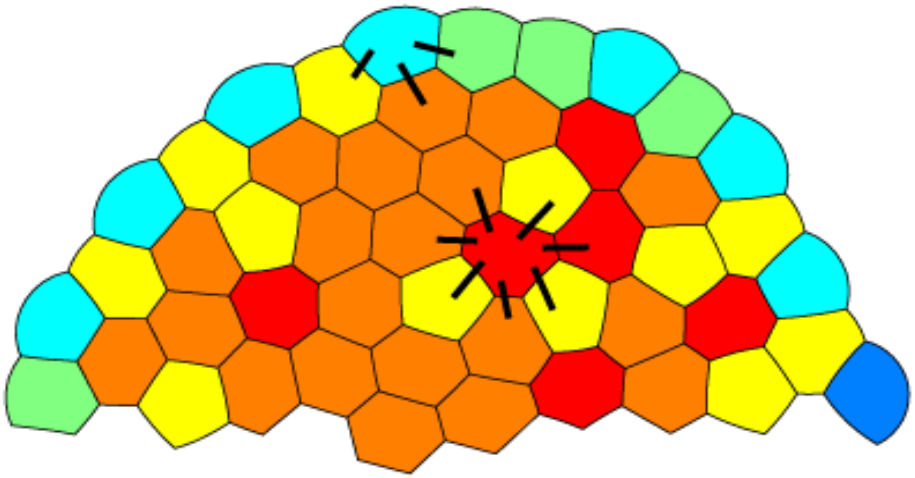
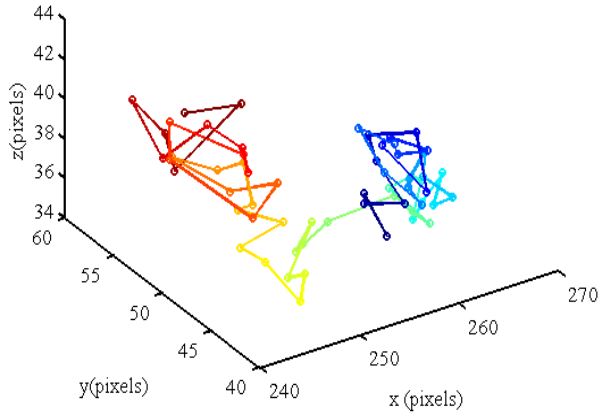
What properties of single cells give rise to the emergent properties of tissues?

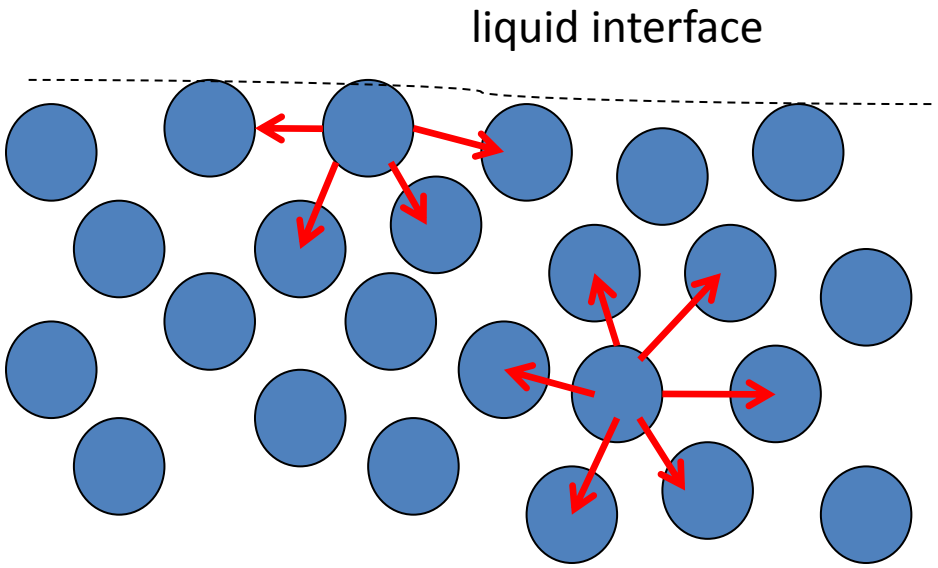
- Part I: Steady state
  - observations: surface tension, cell shapes
  - theory tool: thermodynamics
- Part II: Time-varying
  - observations: viscosity, elasticity, cell nuclei tracking
  - theory tool: dynamics, kinetics





Part I: Surface tension in steady state

for normal fluids, surface tension is the difference in average energy (Δ W) between a surface molecule and an interior molecule, times the number of molecules per unit surface area



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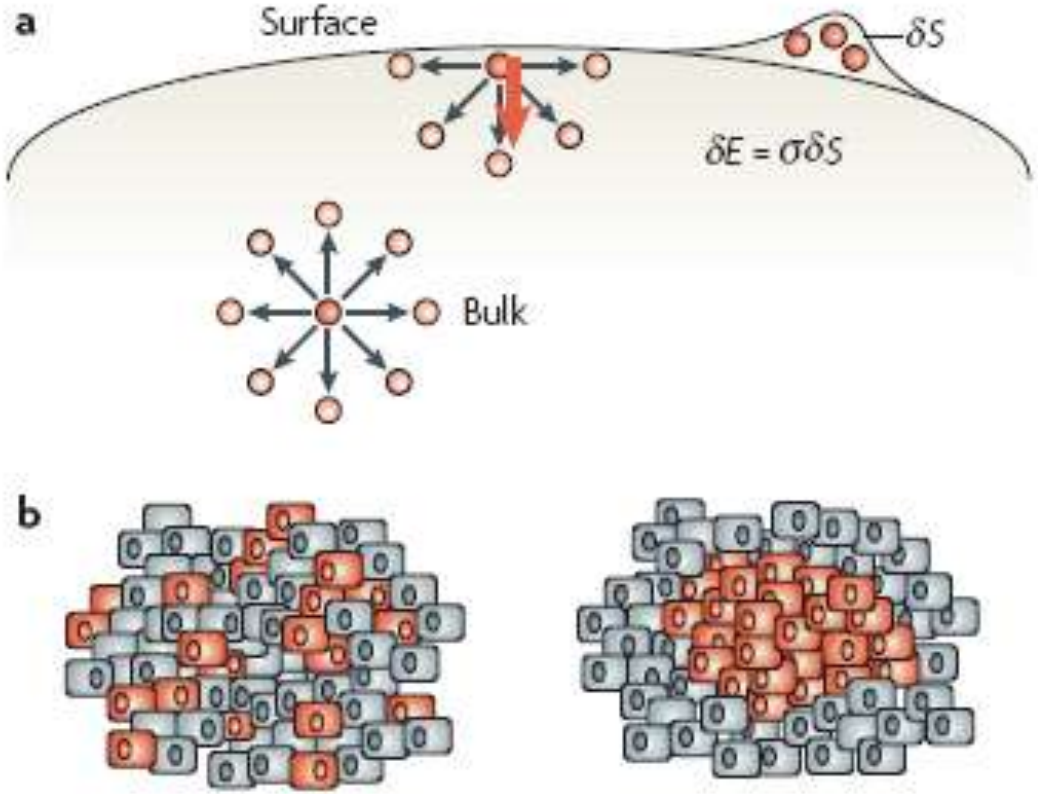

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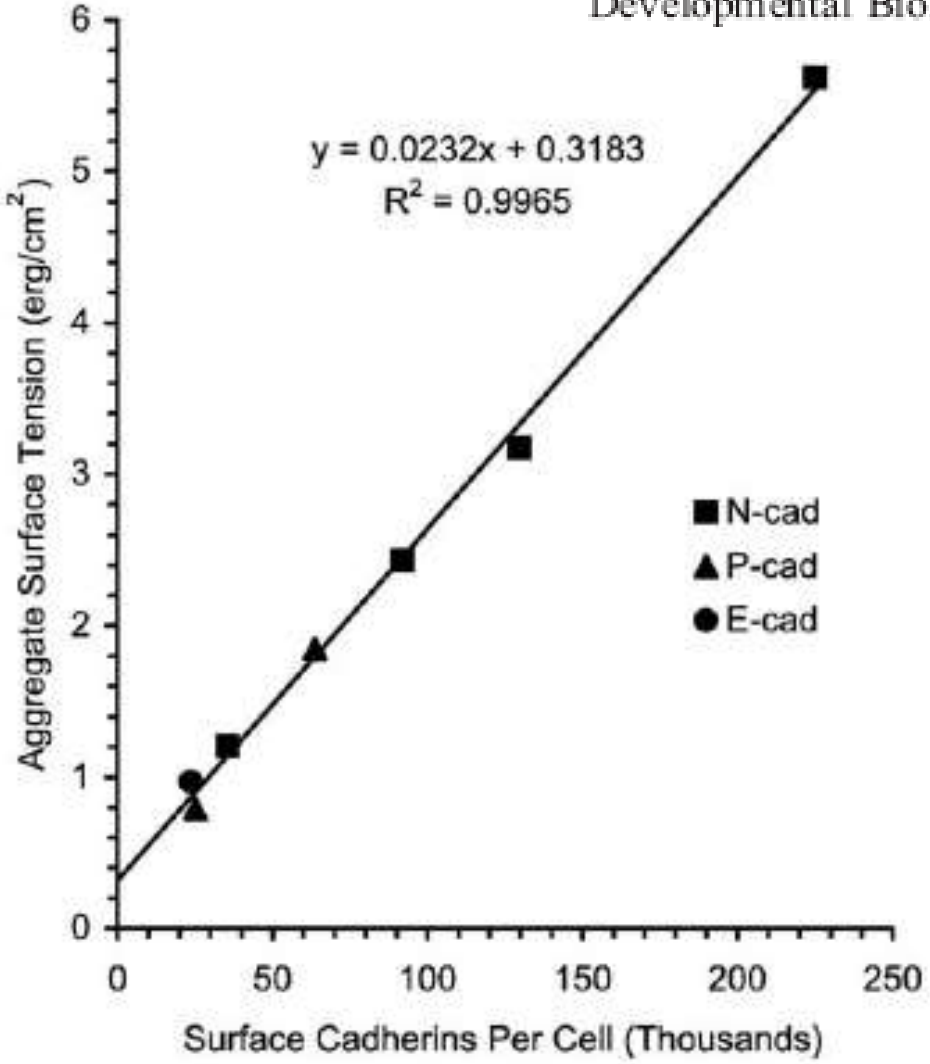
Lecuit and Lenne

The differential adhesion hypothesis: a direct evaluation

Ramsey A. Foty[a], Malcolm S. Steinberg[b,*]

Developmental Biology 278 (2005) 255–263

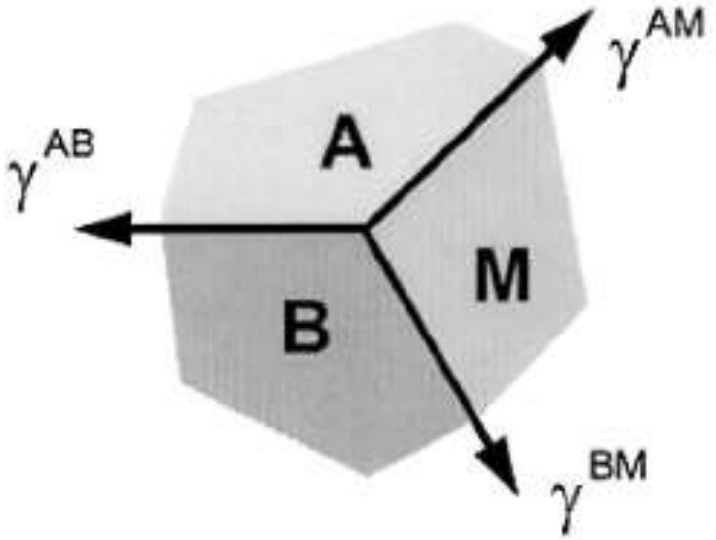


Differential interfacial tension (DITH) or surface contraction hypothesis

Harris, Brodland

"interfacial tensions …ultimately lead to specific patterns of cell rearrangement"

i.e., cortical tension should matter



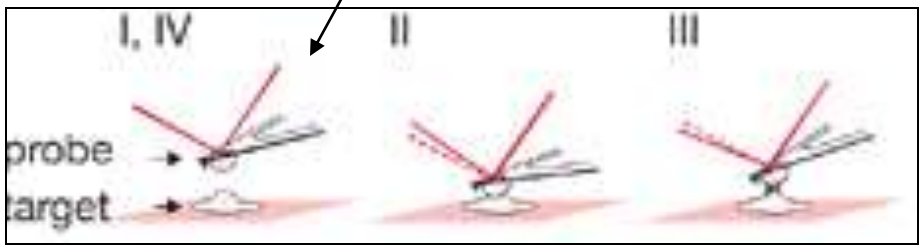
Brodland

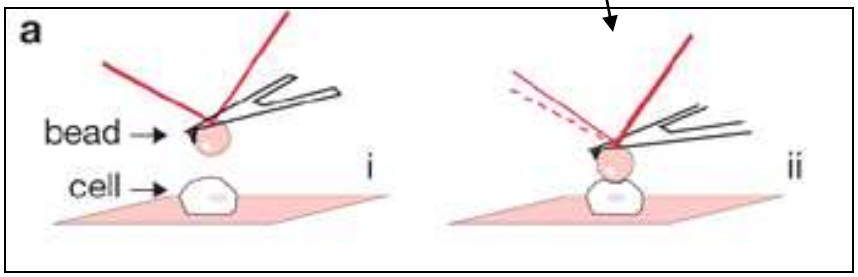
Appl Mech Rev vol 57, no 1, January 2004

Experiment: different tissues

Ordering, highest to lowest

surface tension	"adhesion"	"cortical tension"
1.Ecto	1. Meso	1.Ecto
2.Meso	2. Endo	2.Meso
3.Endo	3. Ecto	3.Endo





Krieg et al, Nature Cell Biology 10, 429 - 436 (2008)

What specifies surface tension?

1) only the adhesive interactions between individual cells?
2) or does cortical tension matter?

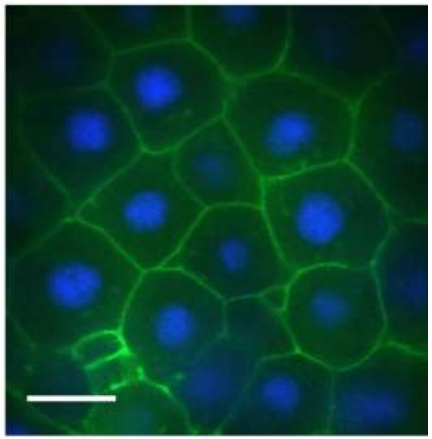
What is the role of geometry (cell shapes)?

“Steady-state” cell model: what mechanical forces act to generate steady state cell shapes?

1. Surface adhesion: cadherins
2. Active cortical tension: myosin motors (Joanny, Prost et al)
3. Bulk effects: fluid resists dialation/ compression, cytoskeleton resists shear
4. Cortical elasticity: cytoskeletal networks

Devries et al, Development **131**, 4435–4445 (2004)

Coarse-grained energy: single cell



$$W_{tot} = -\frac{\gamma P_c}{2} + \beta P_T \quad | \quad V = V_0$$

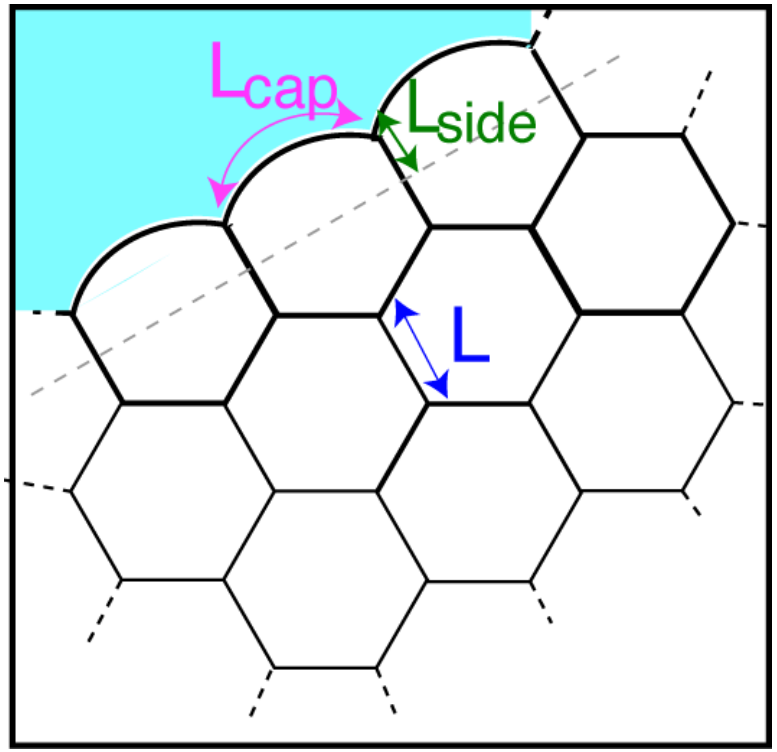
adhesion

cortical tension

incompressibility

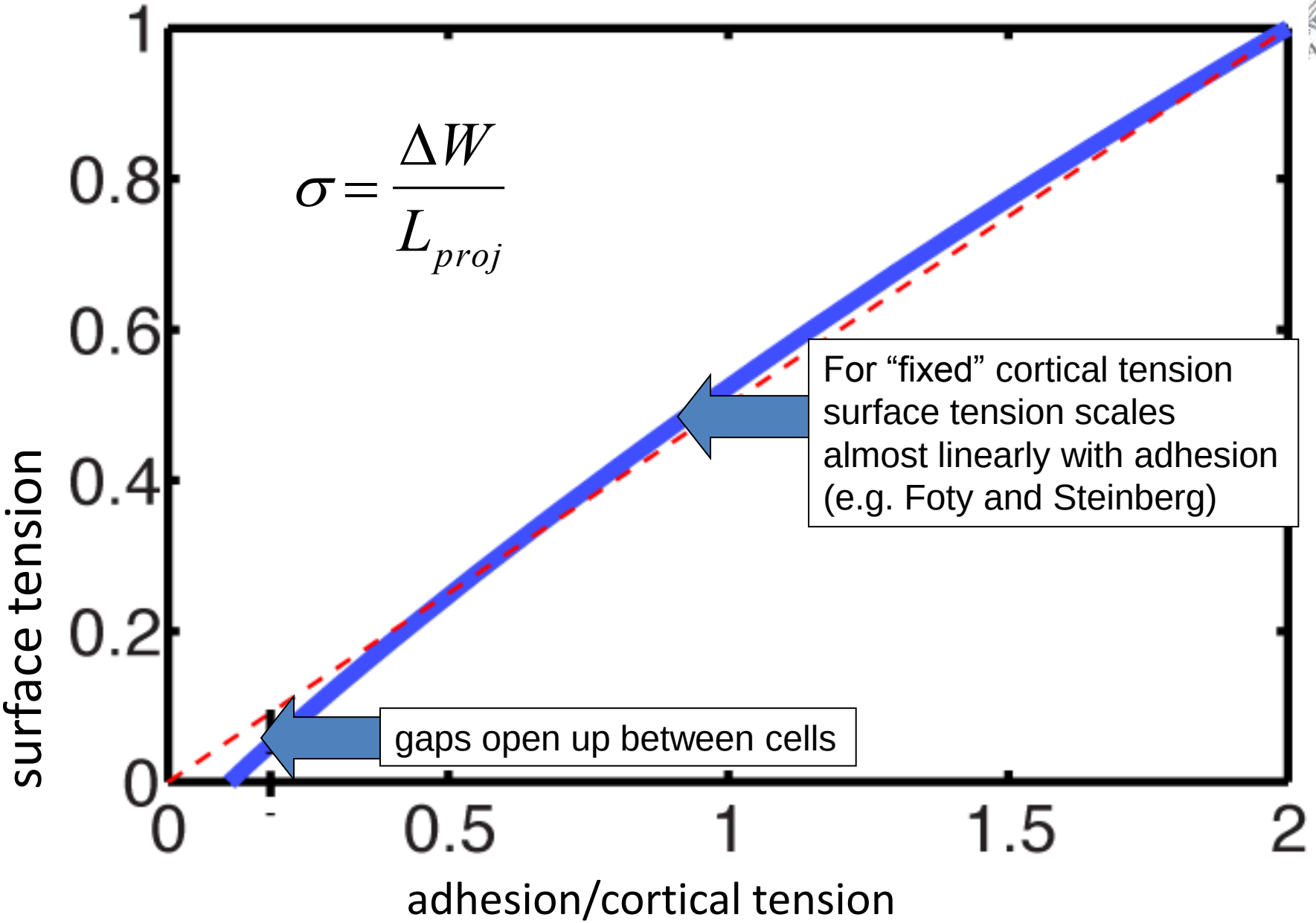
Goal: steady state cell shapes

Find the local minimum energy shapes for a collection of cells subject to constraints

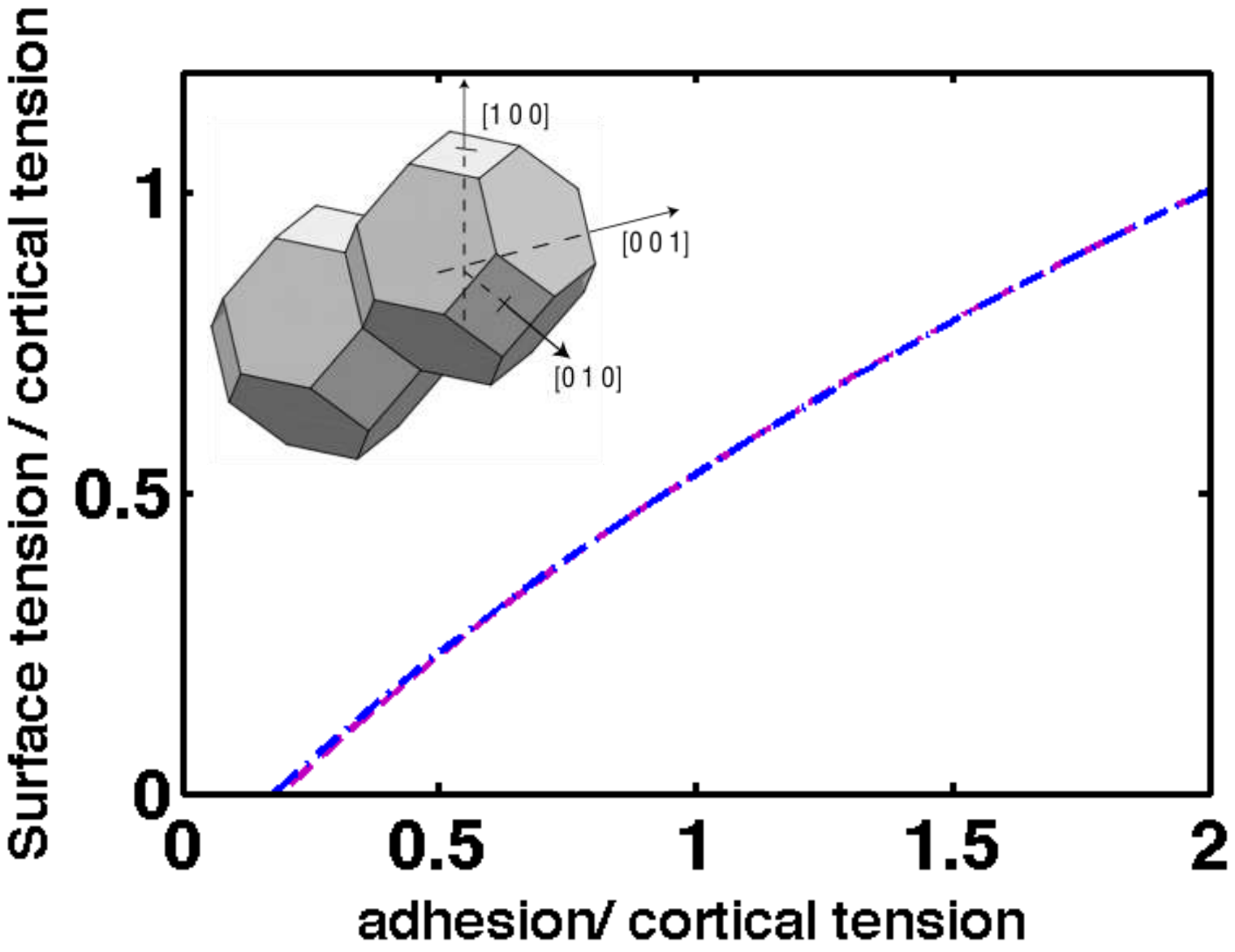


- difficult in general (functional shape derivatives)
- exact solution(!) for 2D ordered packing

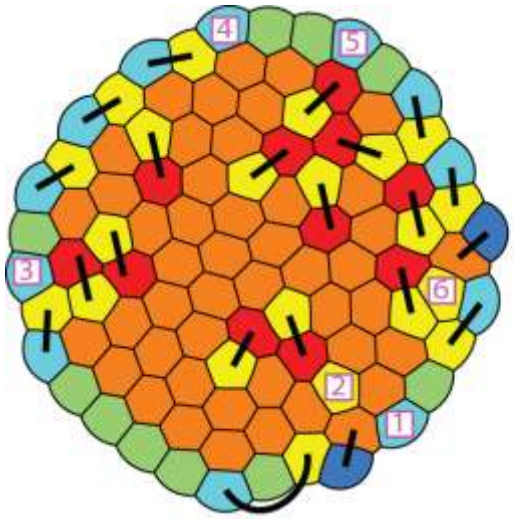
2D- ordered solution

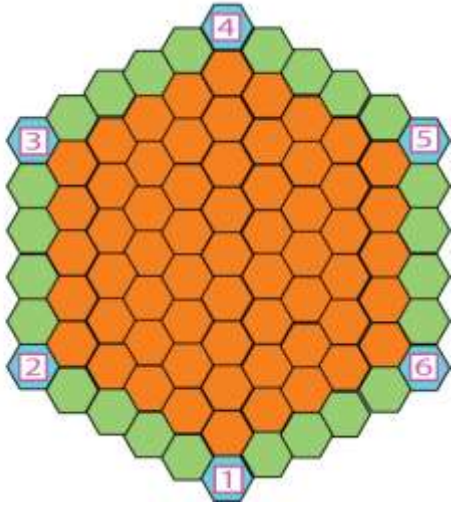


3D- ordered solution



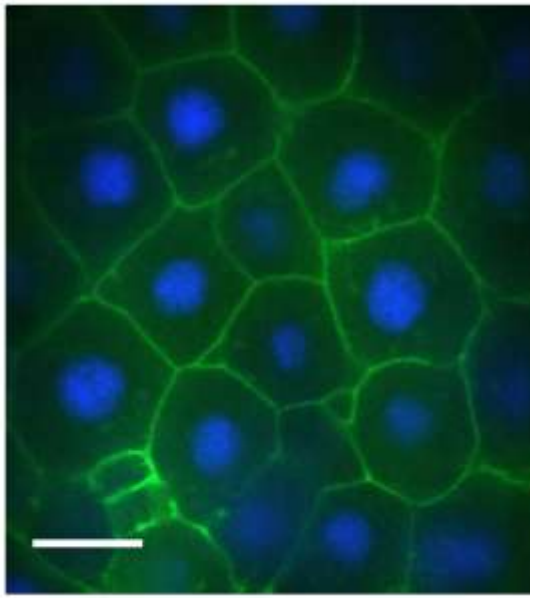
Effects of disorder





Disordered – perimeter has circular symmetry

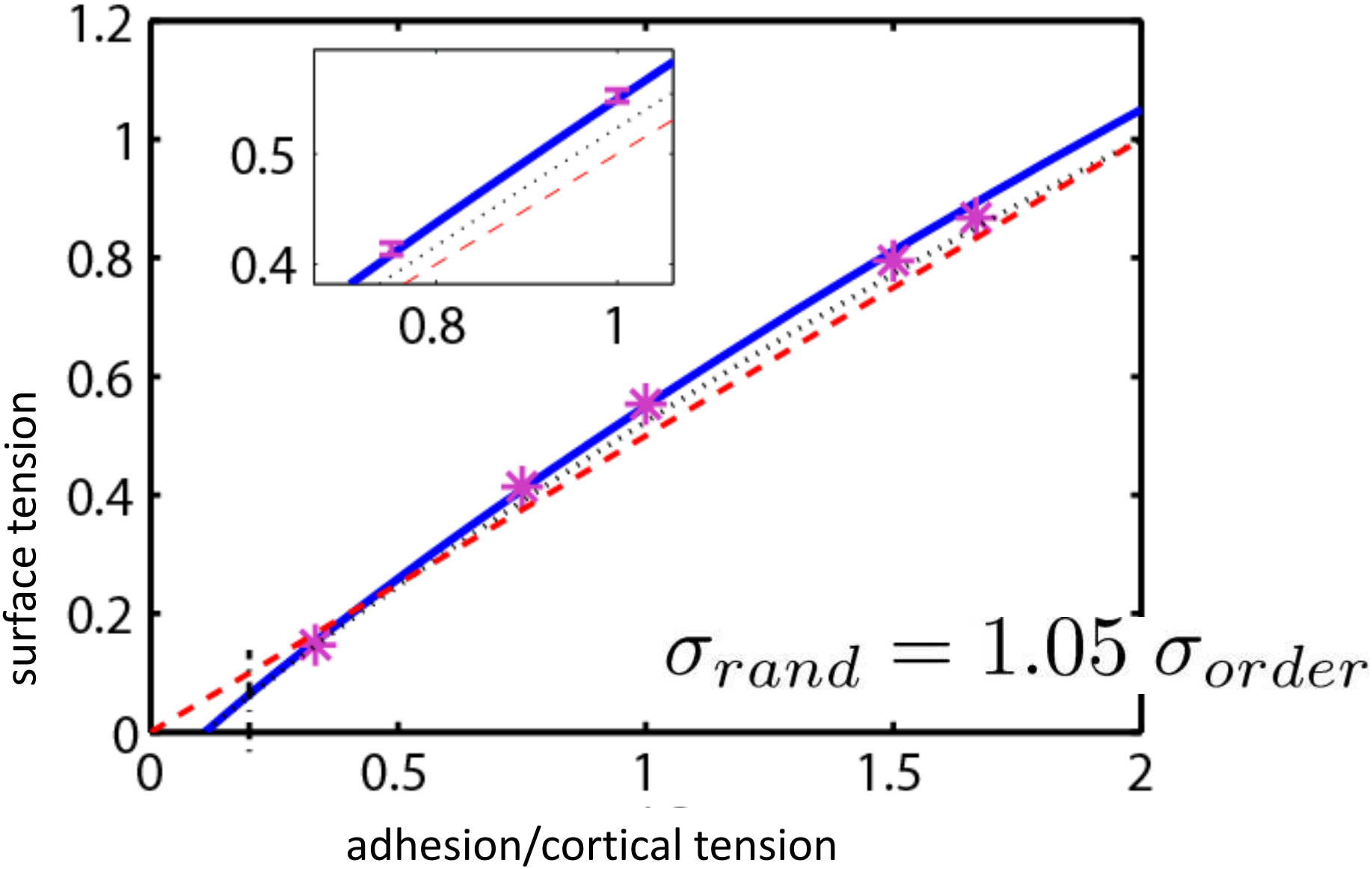
Ordered – perimeter is hexagonal



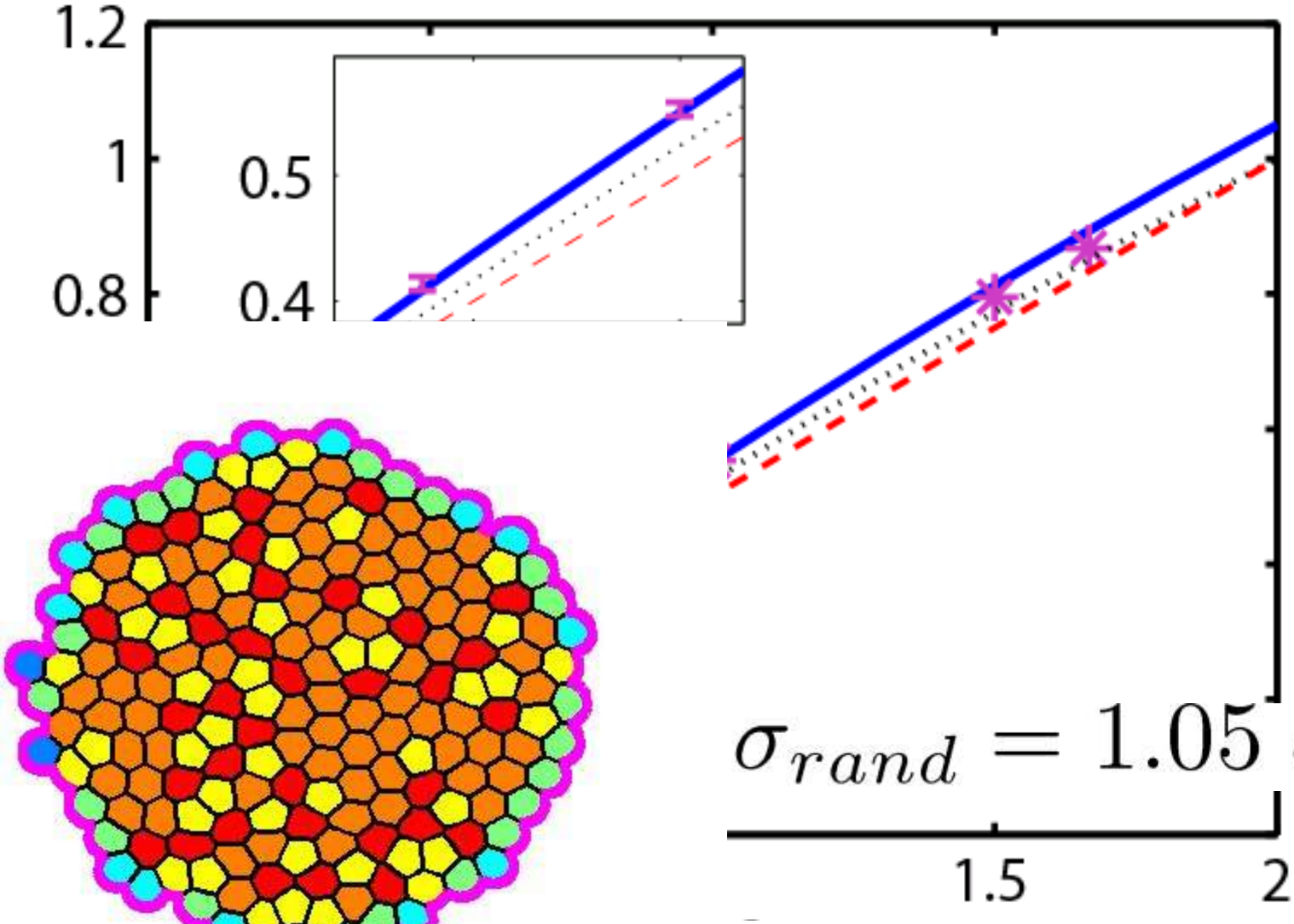
$$\sigma_{disordered} = \frac{L_{hex}}{L_{circ}} \cdot \sigma_{ordered}$$

$$\sigma_{disordered} \approx 1.05 \cdot \sigma_{ordered}$$

disordered surface tension

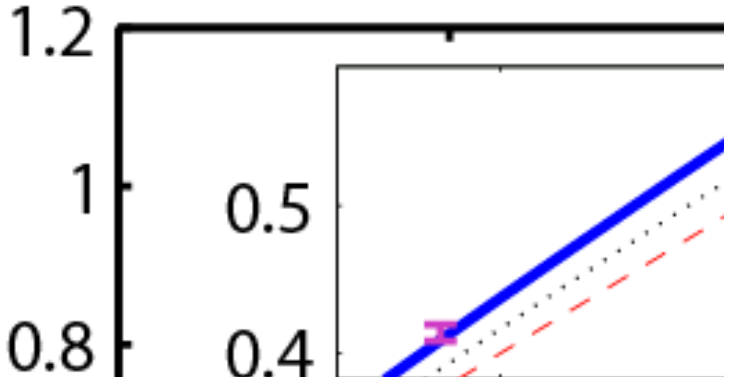


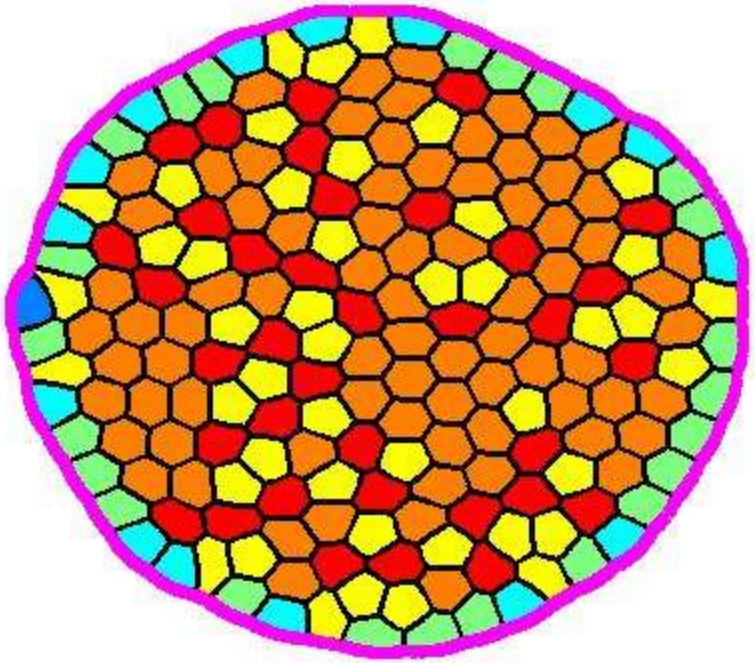
disordered surface tension

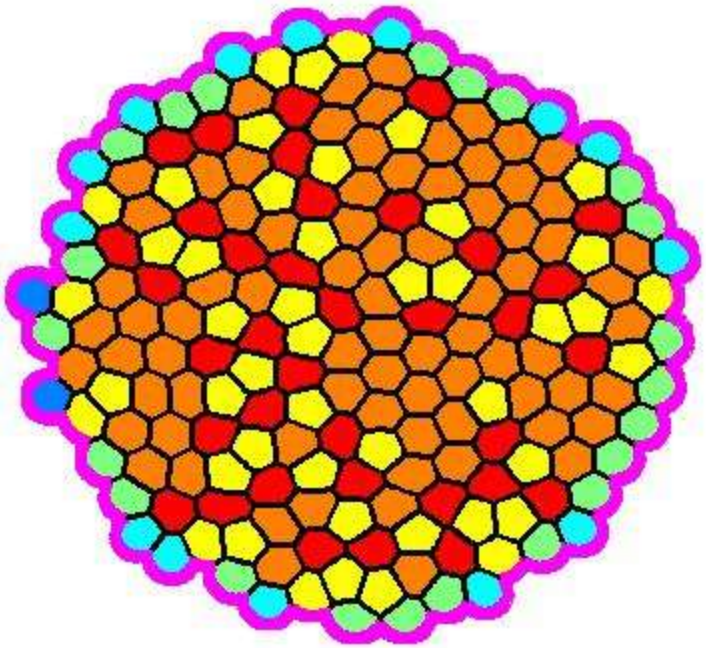


al tension

disordered surfac





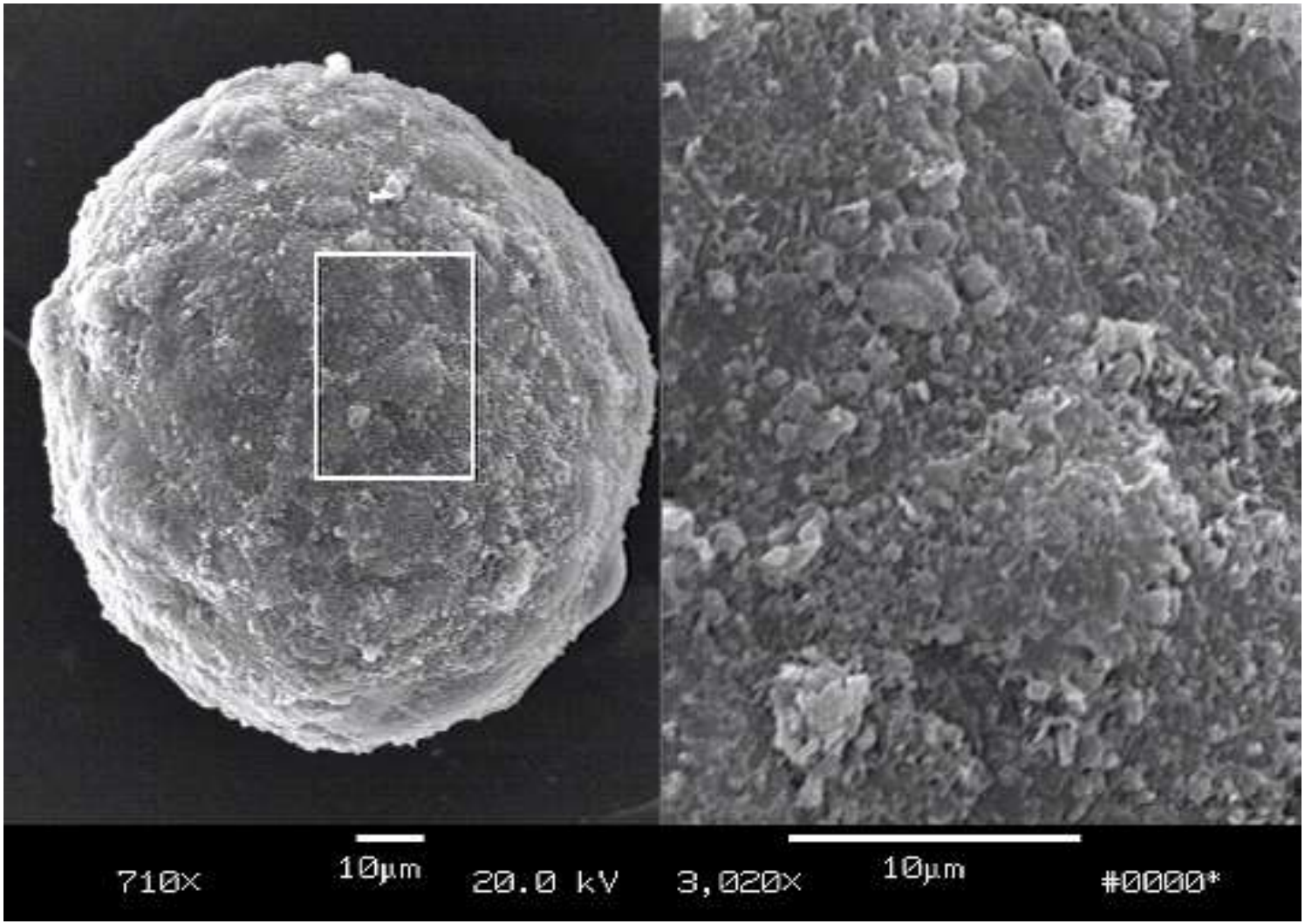


$$\sigma_{rand} = 1.05\,\sigma_{order}$$

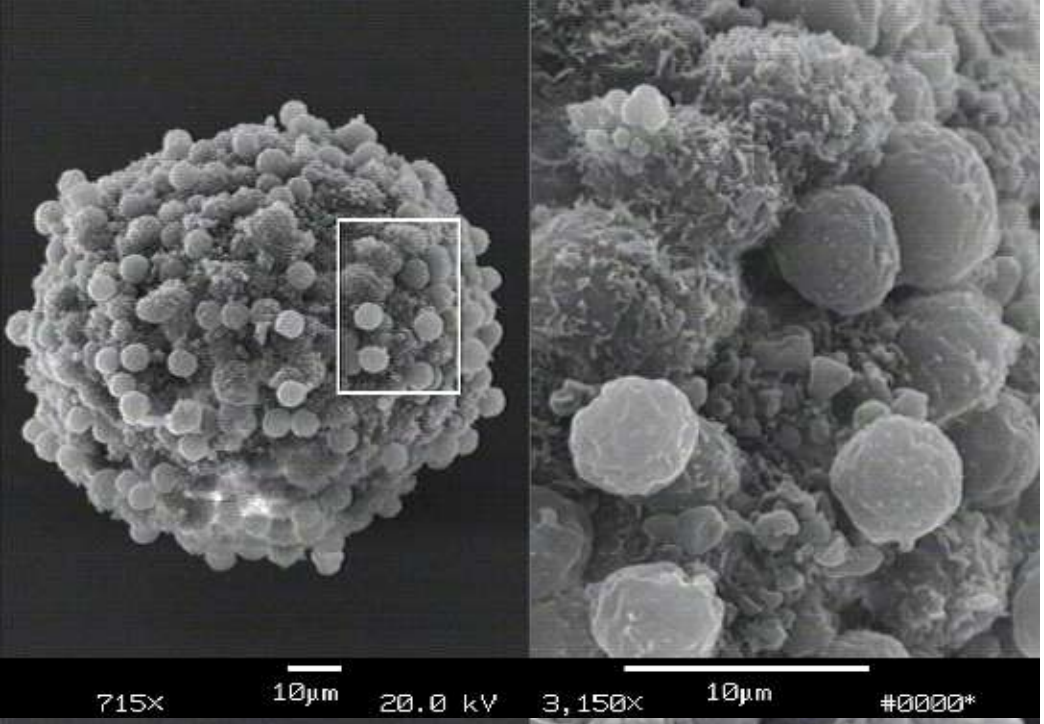
1.5 2

al tension

LP1 cells, SEM, high surface tension



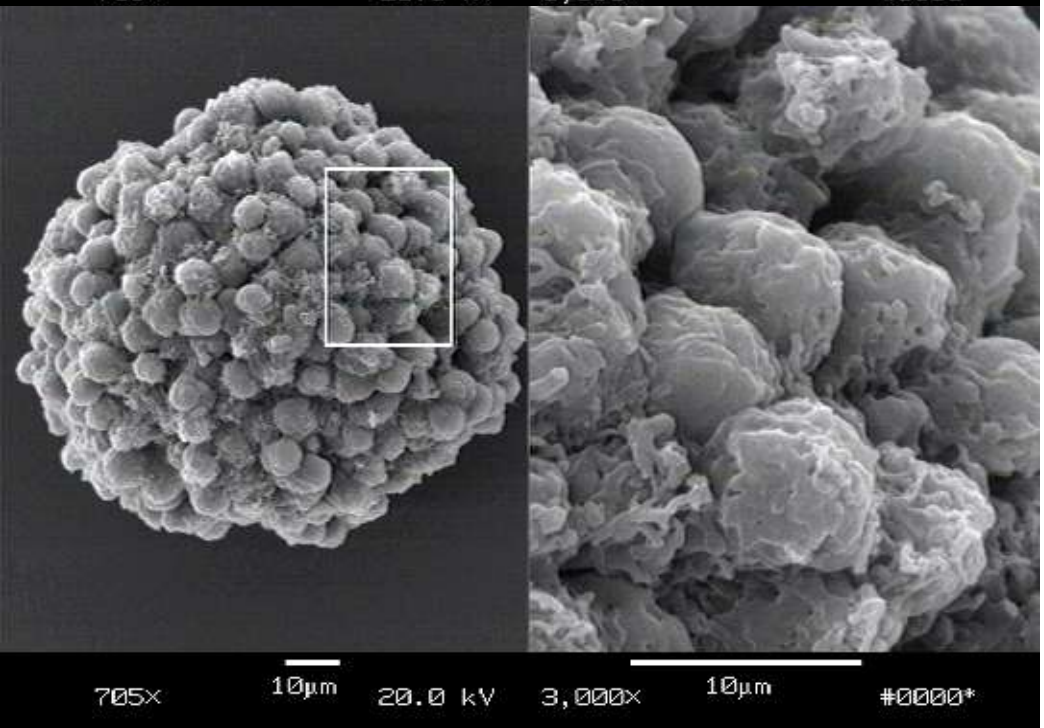
Control:
σ = 3.61
erg/cm^2



Latrunculin A

(prevents g-actin from polymerizing)

$\sigma = 0.15$ erg/cm^2



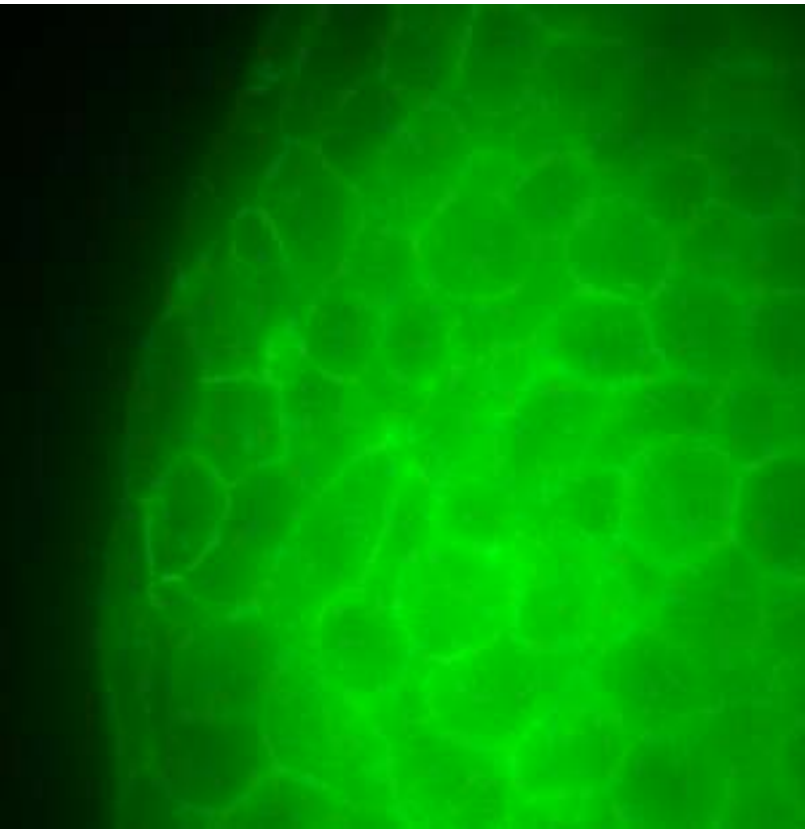
Cytochalisin D

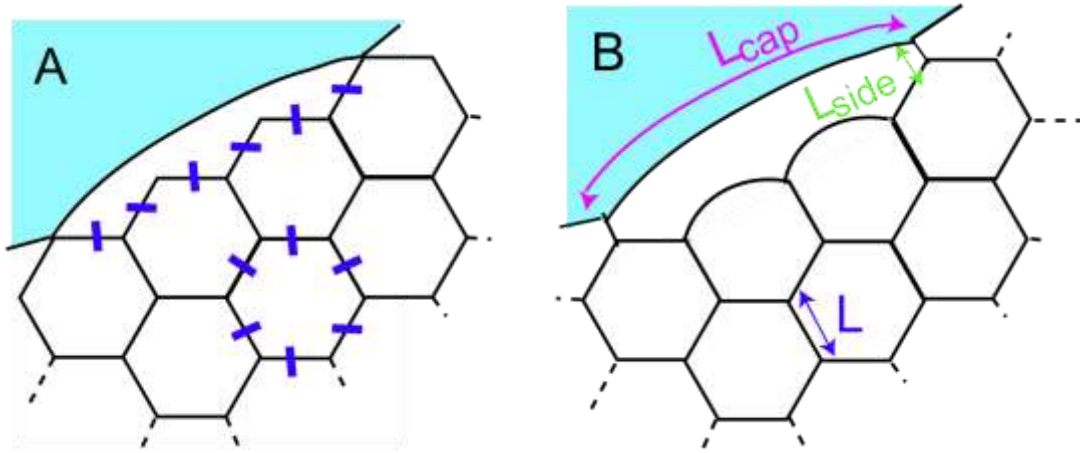
(caps growing end of actin filaments)

$\sigma = 0.31$ erg/cm^2

For very highly adhesive aggregates, surface cells are surprising

Simple model has an instability when adhesion/cortical tension > 2, and surface cells change their shapes

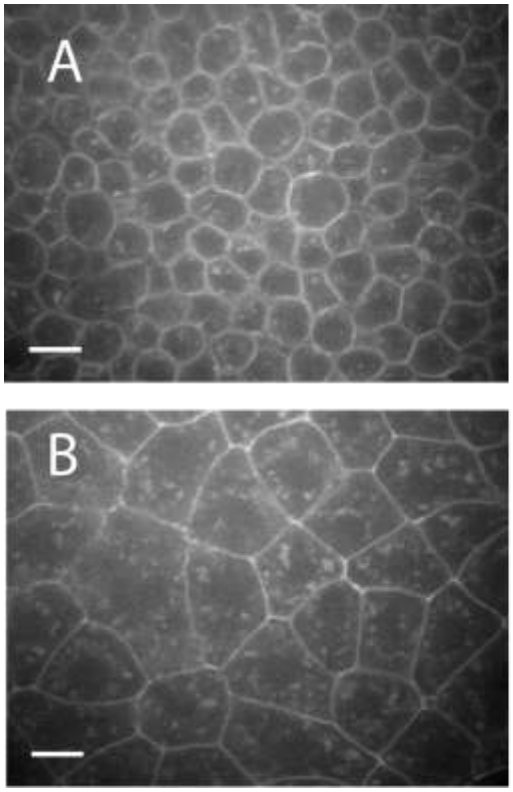


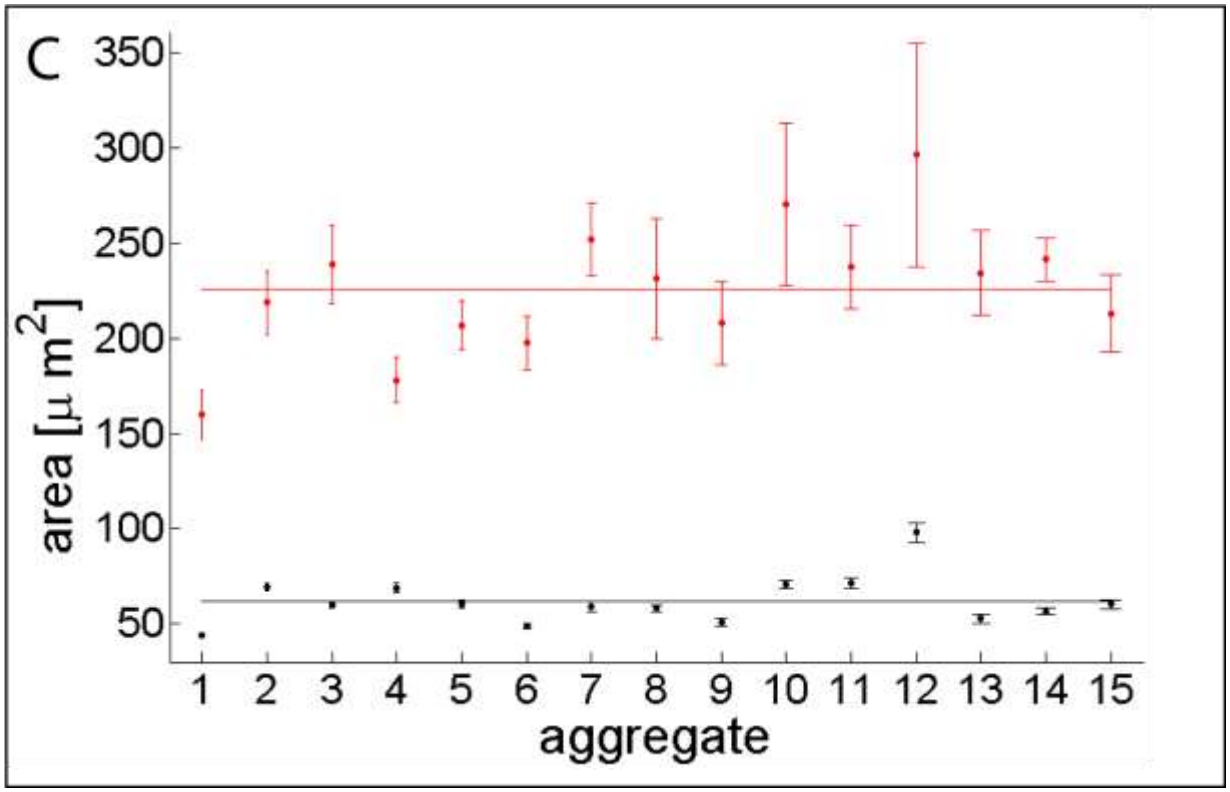


Calculate “projected area” of surface cells using an extension of the model

- 2D ordered packing: surface cells must cover 3 cells below.
- 3D ordered packing (2 facets): 3.7

Data:





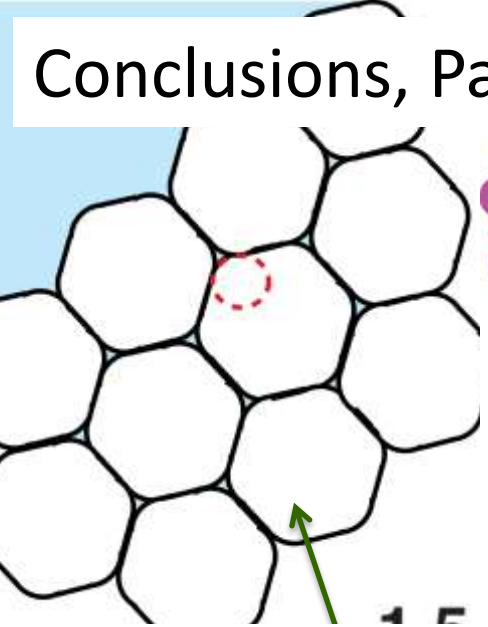
Surface cells have projected areas

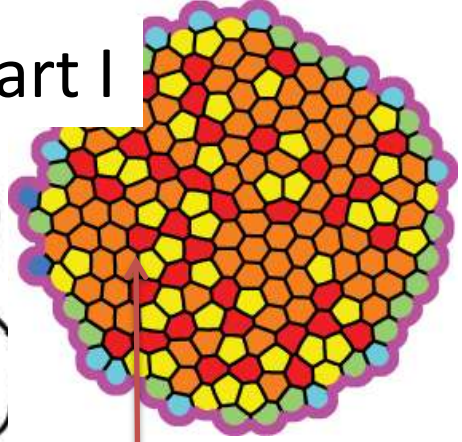
3.7 ± 0.4 times greater than interior cells

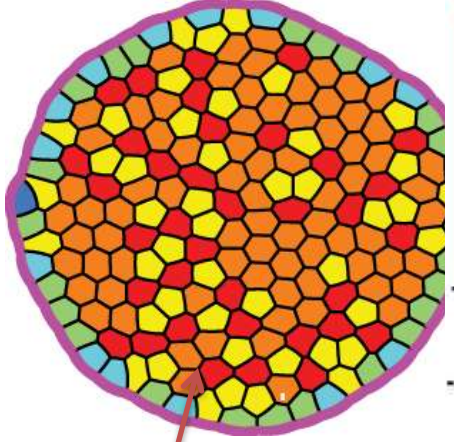
Model independent observation:

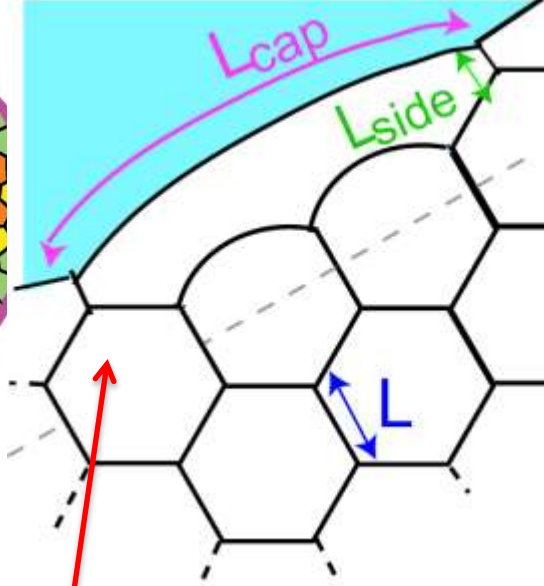
- DAH: just as in fluids, surface cells make fewer neighbor contacts
- If surface cells stretch to make the same contact surface area as bulk cells, then there is no difference in their number of adhesive contacts
- In this regime the DAH, as stated, can not be correct.

Conclusions, Part I

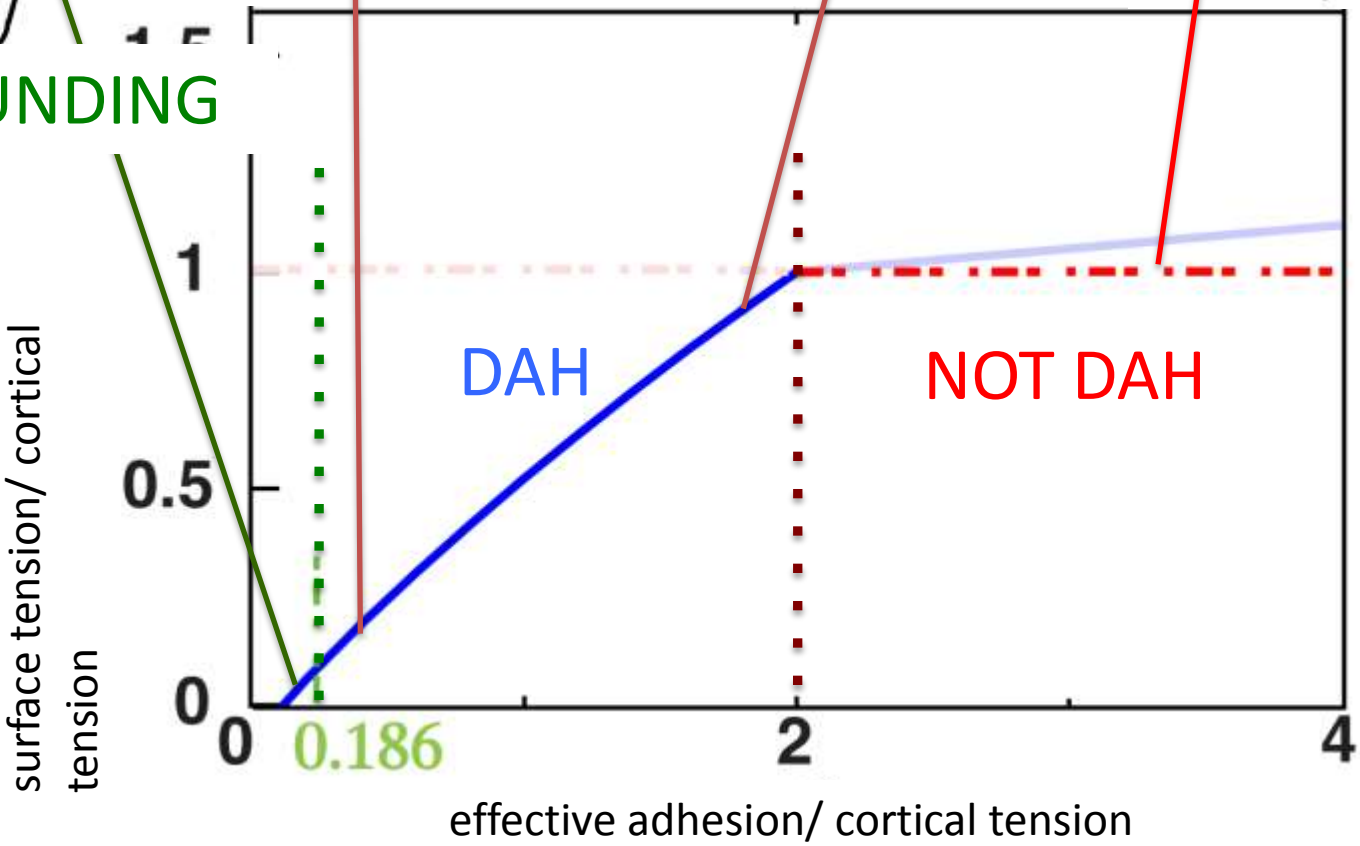








ROUNDING



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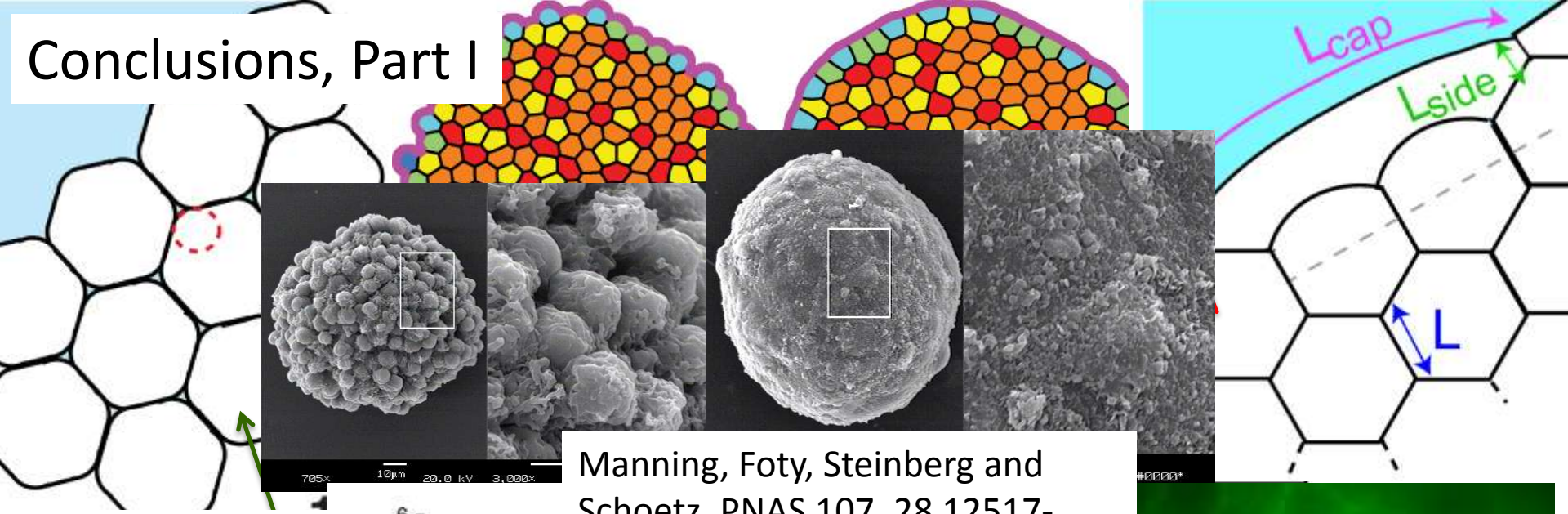
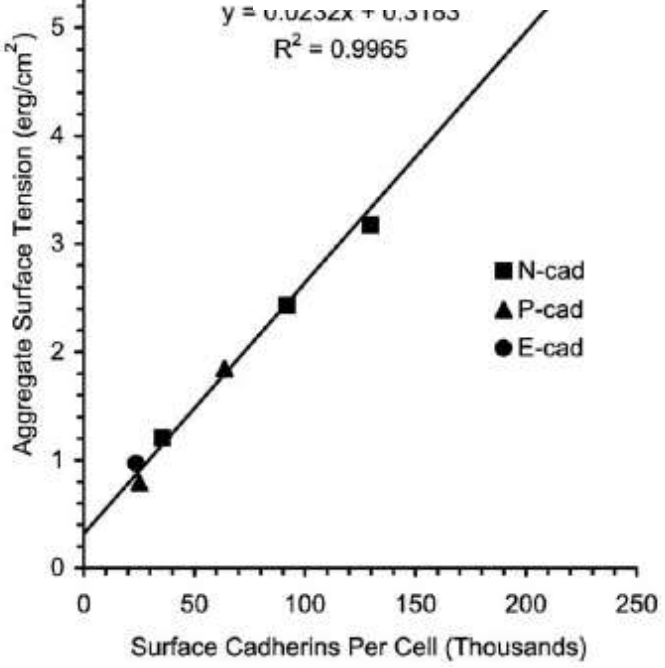
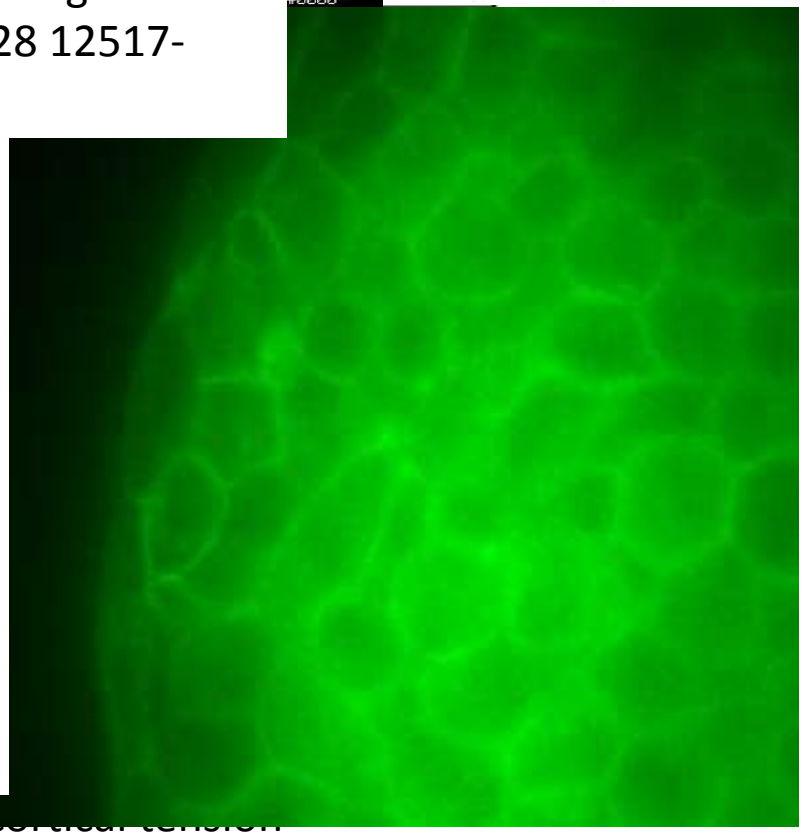


Manning, Foty, Steinberg and Schoetz. PNAS 107, 28 12517-12522 (2010)

ROUNDIN



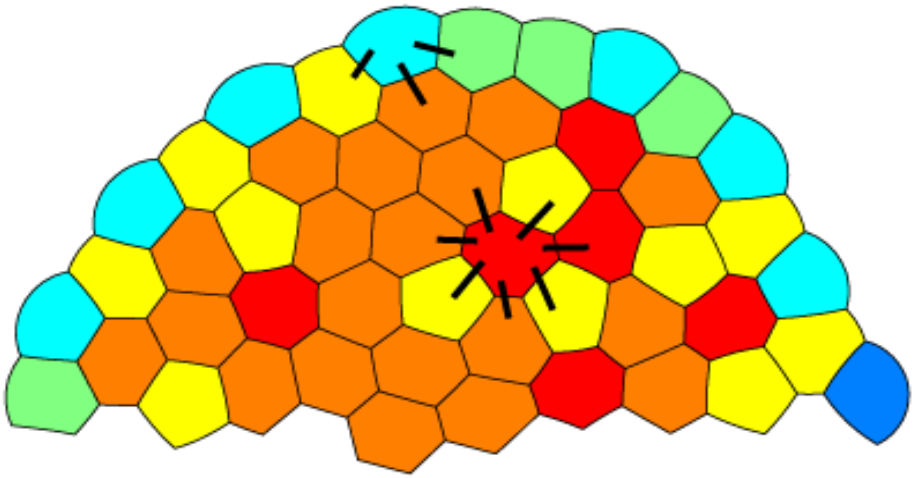
surface tension/ cortical tension



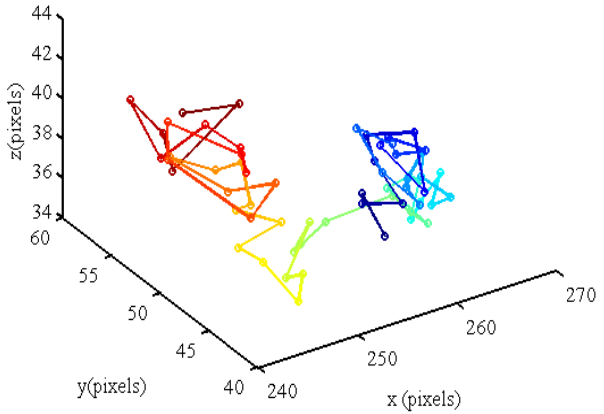
effective adhesion/ cortical tension

What properties of single cells give rise to the emergent properties of tissues?

- Part I: Steady state
  - observations: surface tension, cell shapes
  - theory tool: thermodynamics



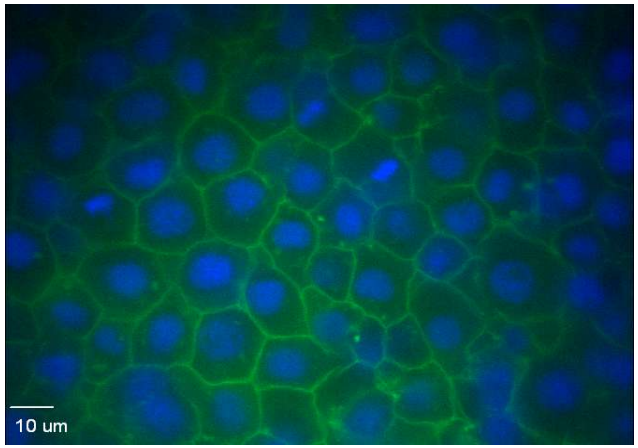
- Part II: Time-varying
  - observations: viscosity, elasticity, cell nuclei tracking
  - theory tool: dynamics, kinetics

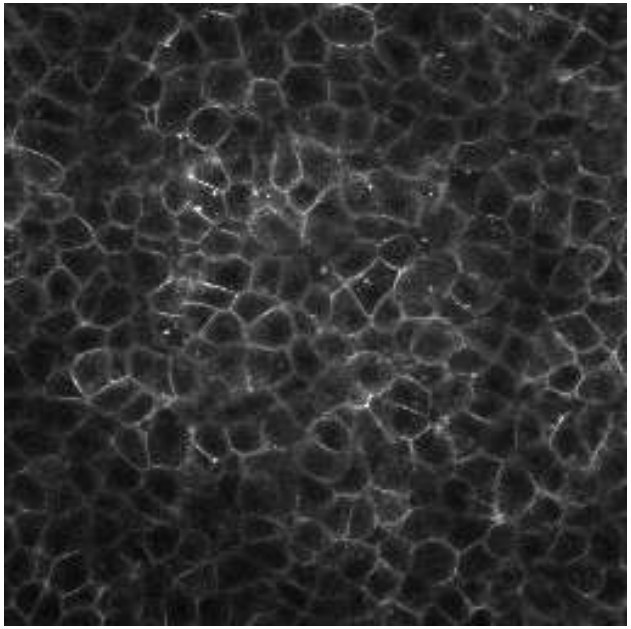


What are the dynamic "material" properties?

Experimental observations:

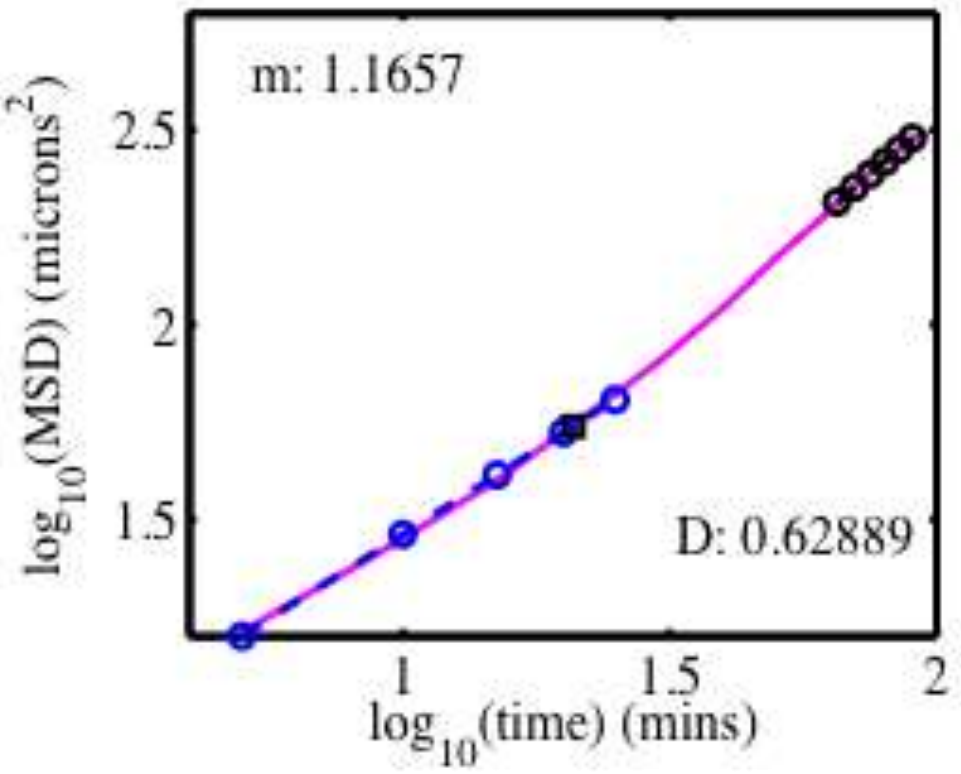
- Structure
  - packing fraction is unity
  - disordered
- Dynamics
  - cell divisions very rare
  - viscoelastic
  - weird boundary conditions for a fluid droplet: no "vapor pressure"
- No existing model is suitable!

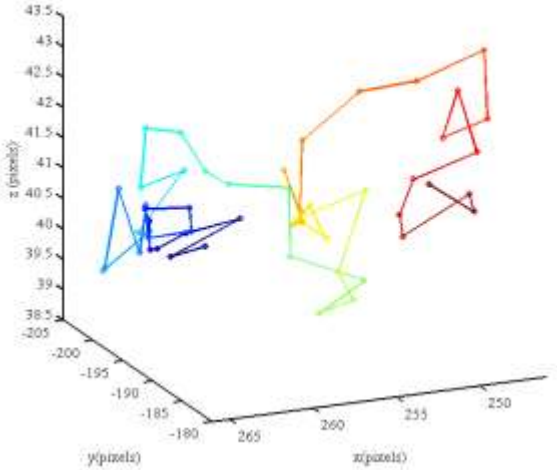


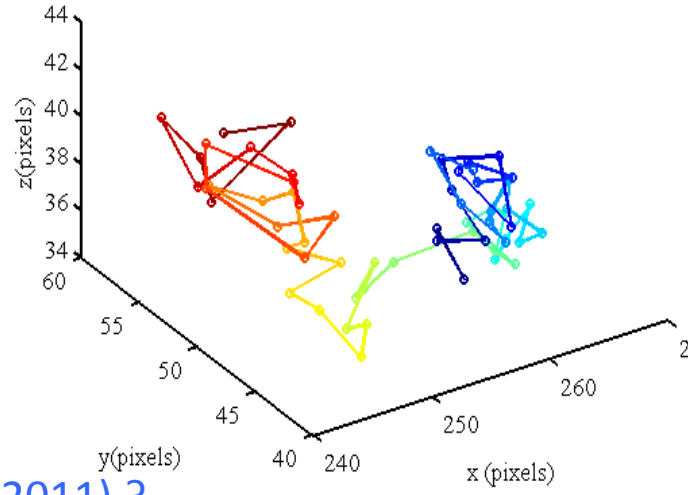


Cell dynamics: nuclei tracking

Ectoderm





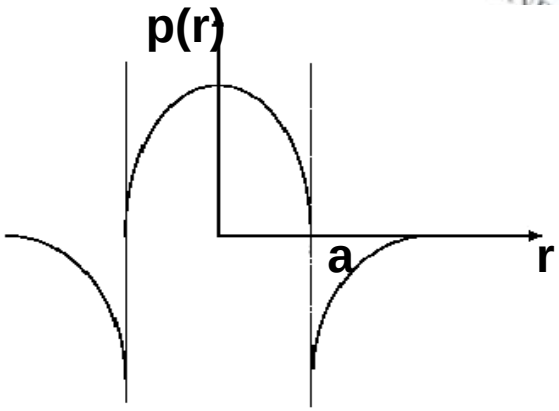


Individual cell tracks
and subdiffusive behavior is reminiscent
of “supercooled” or “glassy” dynamics

real space, 3D analogue of Angelini et al PNAS 108 12 4714 (2011) ?

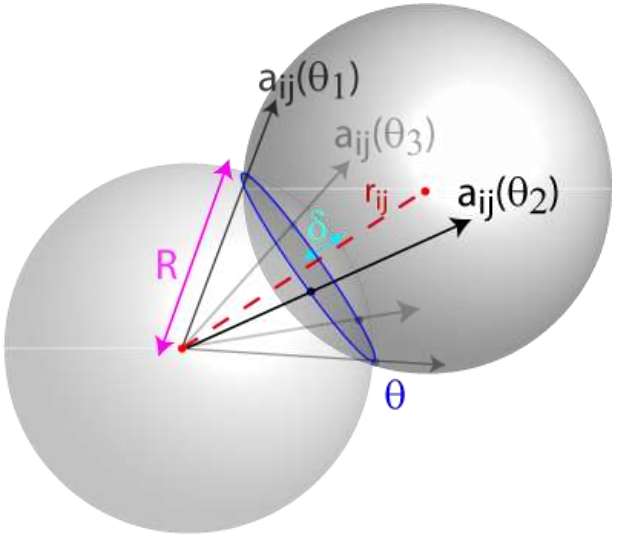
Dynamic tissue model

- Cells resist changes to shape and are adhesive
  - DMT contact mechanics



Derjaguin, B.V., Muller, V.M., Toporov, Y.P., 1975, J. Coll. Interf. Sci., 53, pp. 314-326.

- Cells move past one another by developing protrusions and exerting tension on new contacts
  - leads to a special type of "noise" that is not Gaussian



Dynamic tissue model

- 3 dimensionless parameters (Γ, σ, ξ):

$$\frac{\widetilde{dr_i}}{\widetilde{dt}} = -\sum_j \left[\left(\left(\widetilde{R}_{ij}^{eff} \right)^{1/2} \left(\widetilde{R}_s - \widetilde{d}_{ij} \right) - \widetilde{\Gamma} \widetilde{R}_{ij}^{eff} \right) \hat{r}_{ij} + \widetilde{\sigma}\, \xi\, \hat{a}_{ij} \right]$$

Γ: Adhesion

σ: active force magnitude

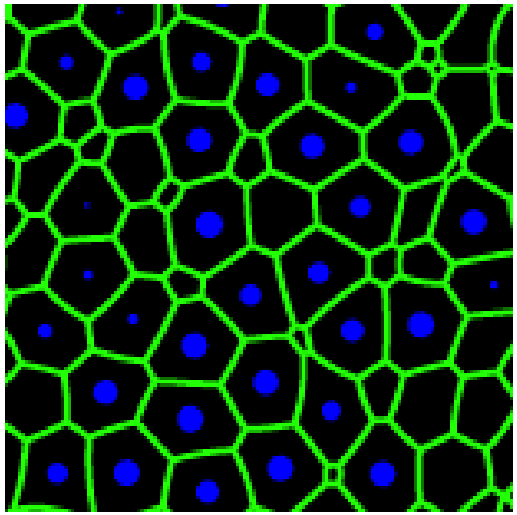
ξ: active force persistence time

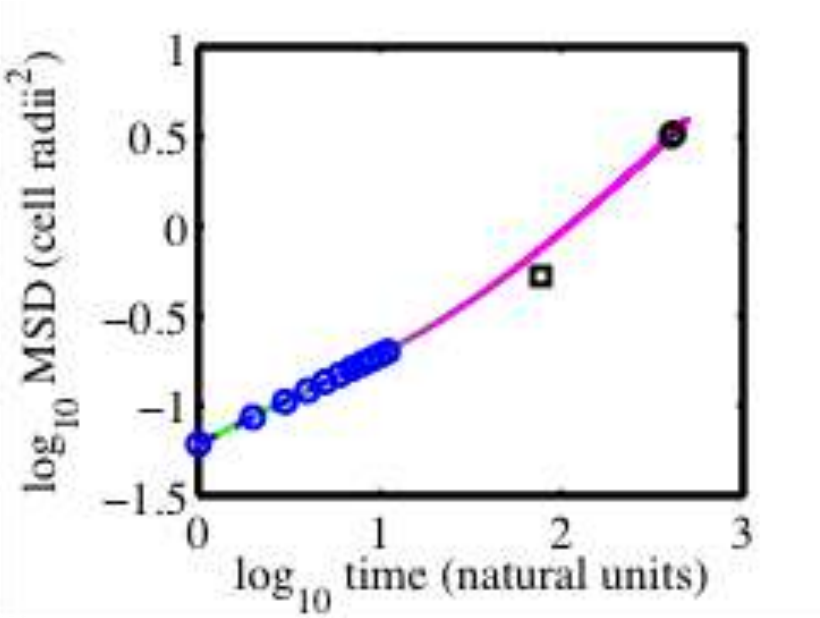
- Captures all qualitative features seen in experiments: rounding up, no vapor pressure, disordered structure

Quantitative calibration:

Four dimensionless observables:

- pf: packing fraction
- m: long-time MSD scaling exponent
- T_c , L_c : crossover from subdiffusive to diffusive behavior
  - time scale t_c : $T_c = (D\, t_c)/R^2$,length scale l_c :$L_c = l_c/R$



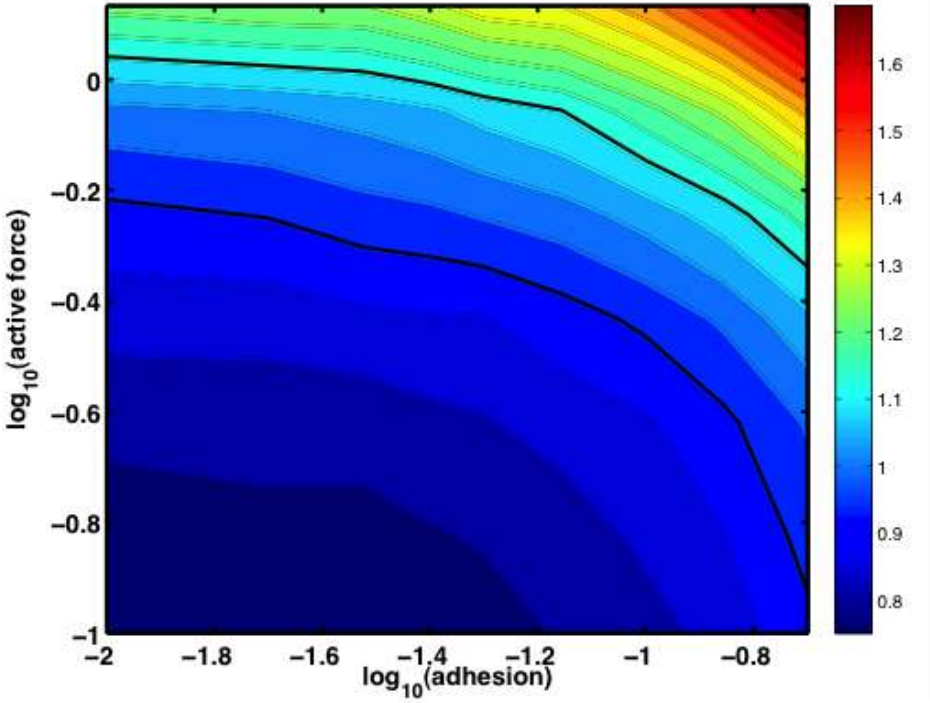


$\Gamma = 0.04$, $\sigma = 0.88$, $\xi = 0.09$

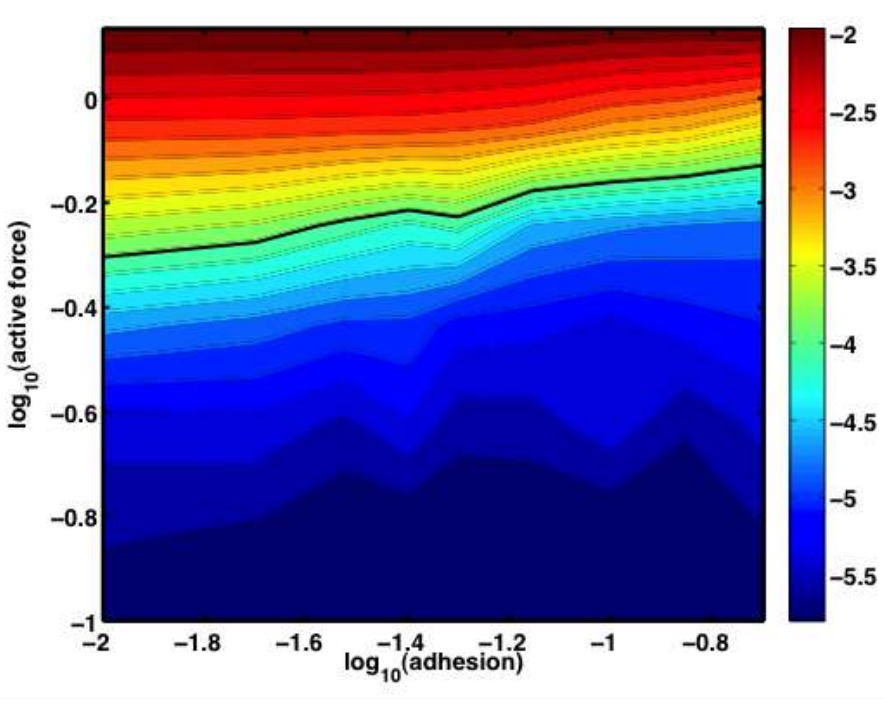
Quantitative calibration

- vary adhesion and active force
- matches experiments: $D > 10^{-4}$, $pf = 1 \pm 0.1$
- Only one point (red) also matches T_c and L_c

1/ packing fraction



log10(diffusion constant)



Quantitative calibration

- vary adhesion and active force
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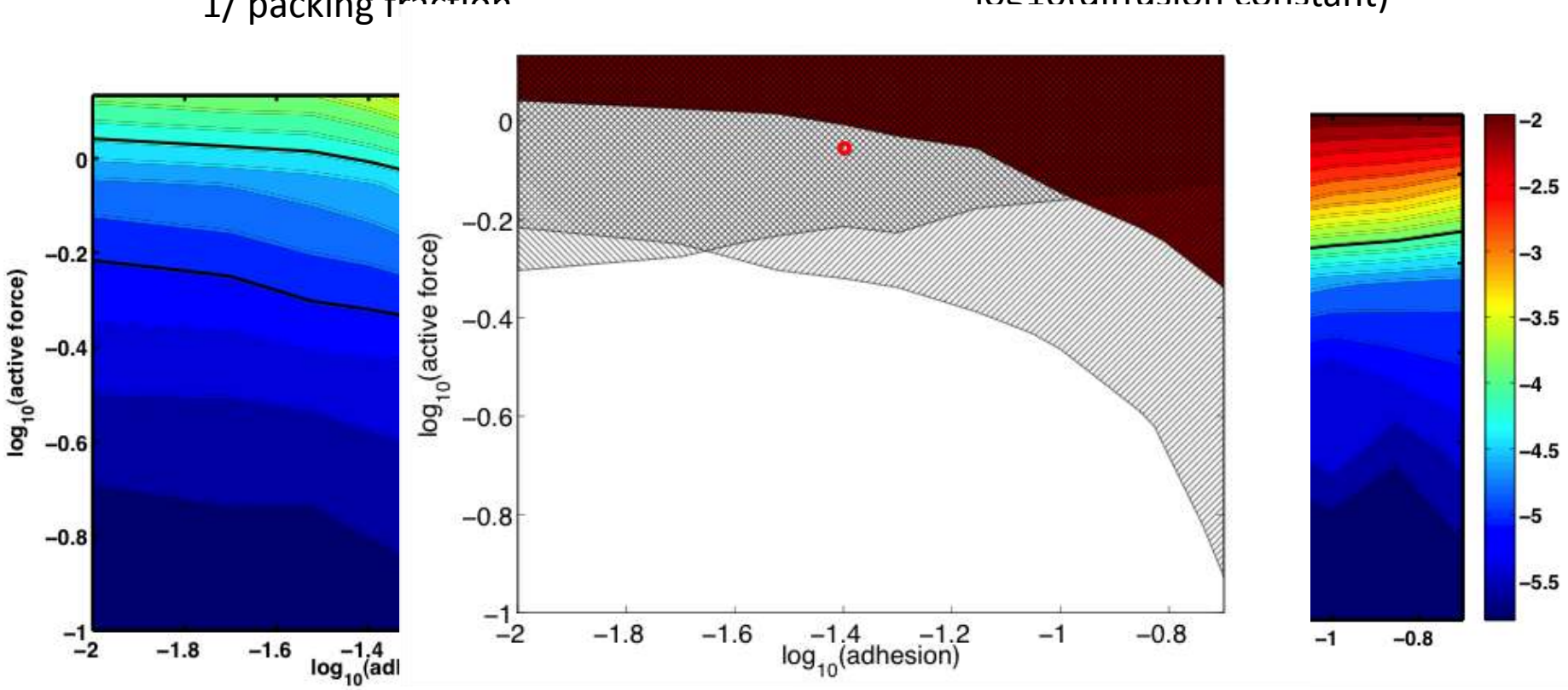
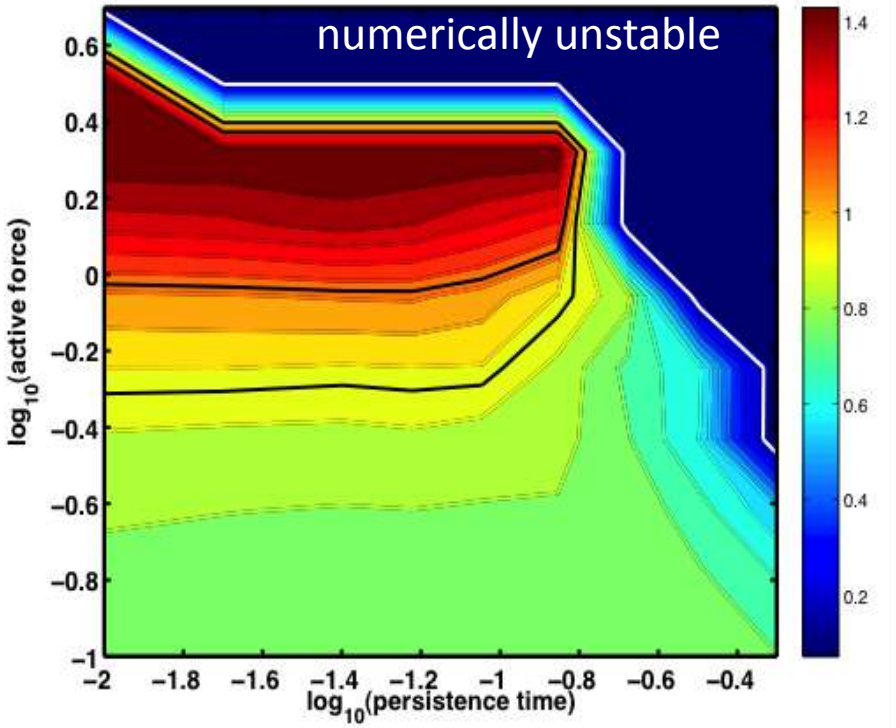
- Only one point (red) also matches T_c and L_c



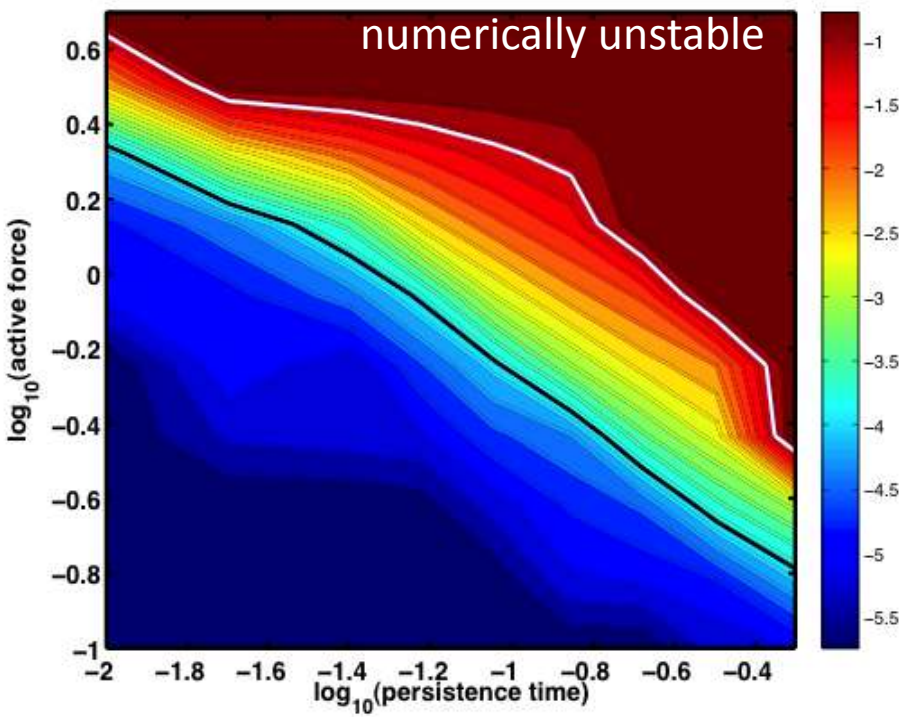
Quantitative calibration:

- vary persistence time, active force, and adhesion (not shown)
- matches experiments: $D > 10^{-4}$, $pf = 1 \pm 0.1$
- Only one point (red) also matches T_c and L_c

1/ packing fraction

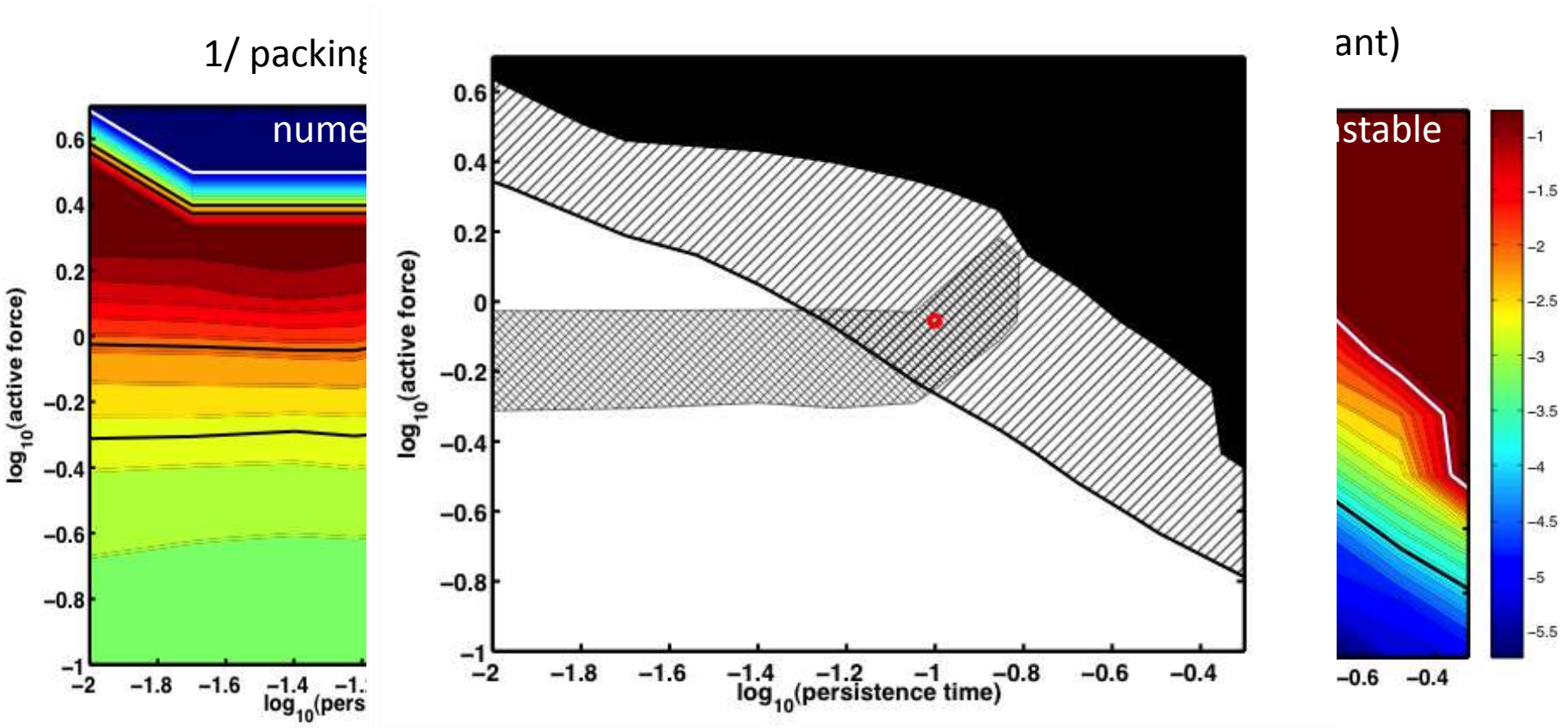


log10(diffusion constant)



Quantitative calibration:

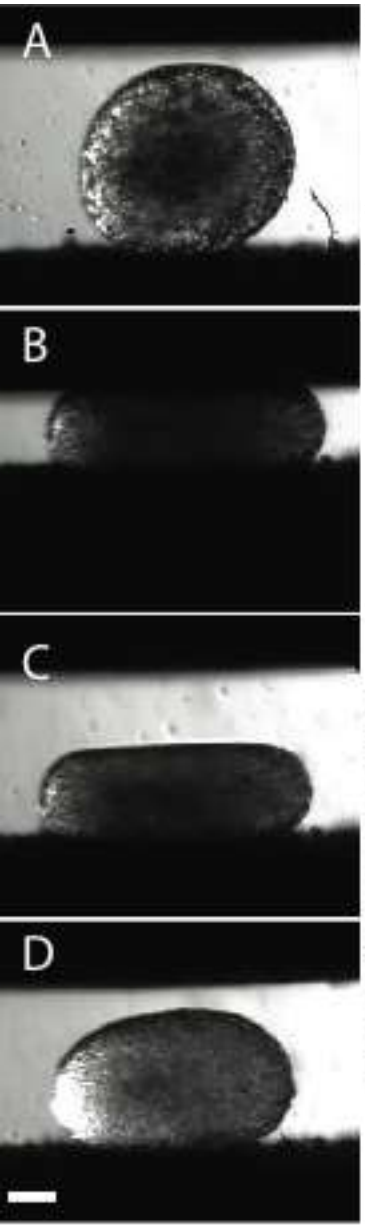
- vary persistence time, active force, and adhesion (not shown)
- matches experiments: $D > 10^{-4}$, $pf = 1 \pm 0.1$
- Only one point (red) also matches T_c and L_c

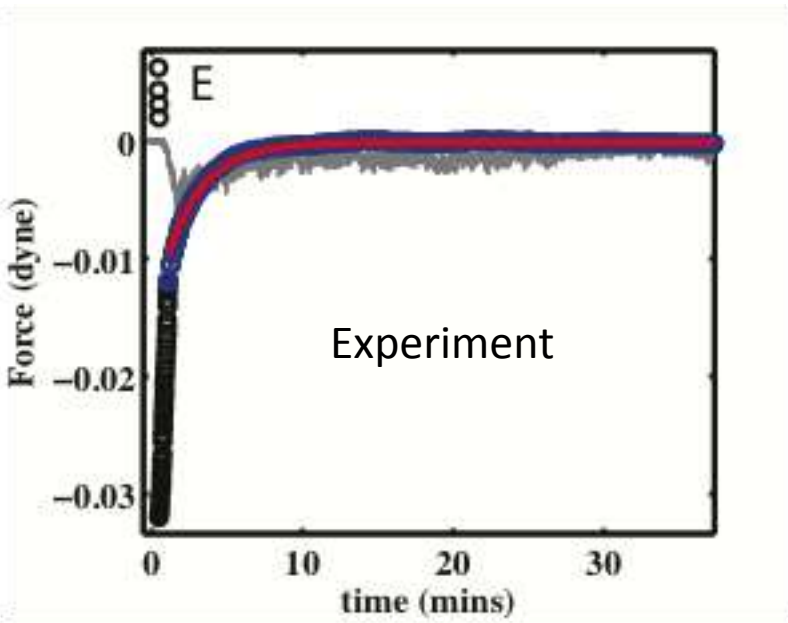


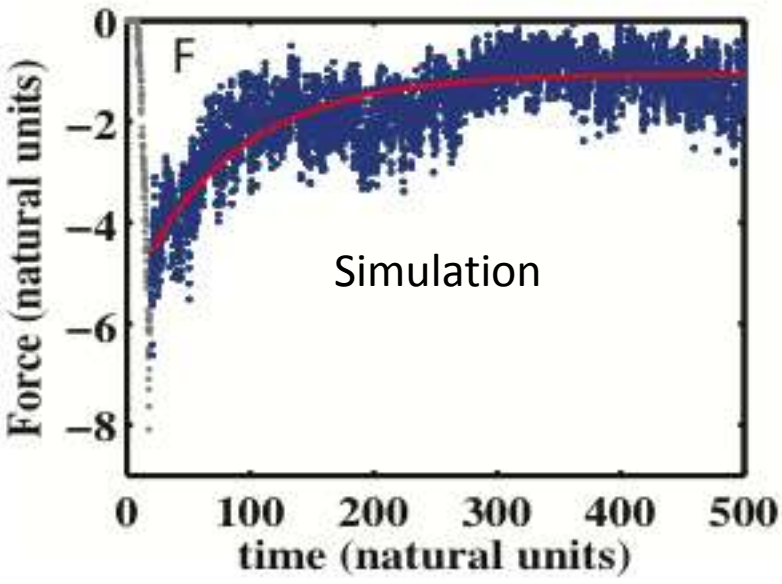
Converting back to real units

- R: average cell radius for simulations
  - 8 microns, from nuclei tracking data
- τ: natural time units for simulations
  - 8 seconds, from long-time diffusion constant for nuclei tracking data (D = 0.62 microns/min)
- F: natural force units for simulations
  - 3×10^{-4} dyn, from initial elastic response in tissue compression experiment (Y = 50 Pa)

Tissue compression predictions?

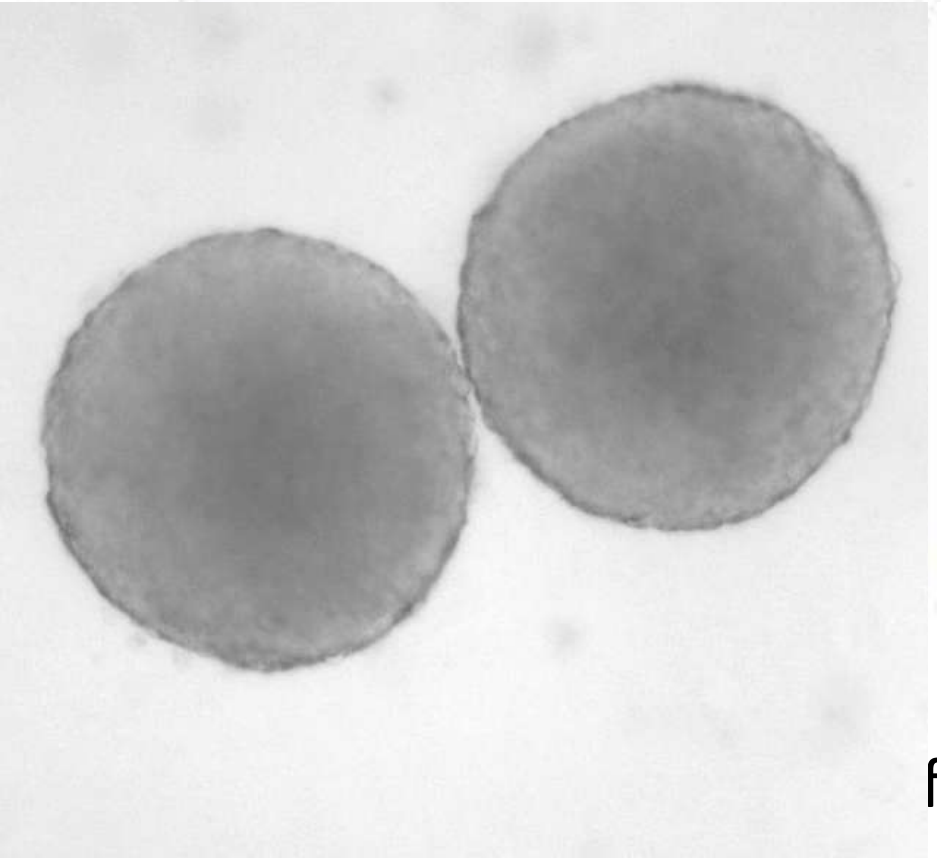


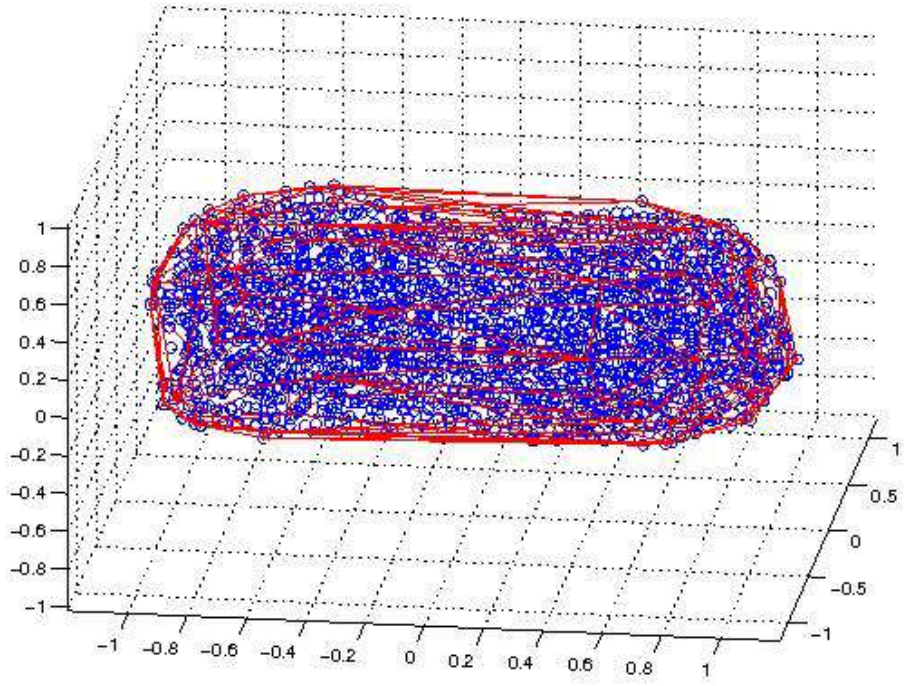




- Qualitative behavior identical (GOOD)
- No fit parameters for viscous relaxation:
  - experiment: 8 min
  - simulation: 11 min
  - GOOD
- But surface tension off by a factor of 30!
  - BAD
  - but, we'll come back to this!

Fusion assay predictions?

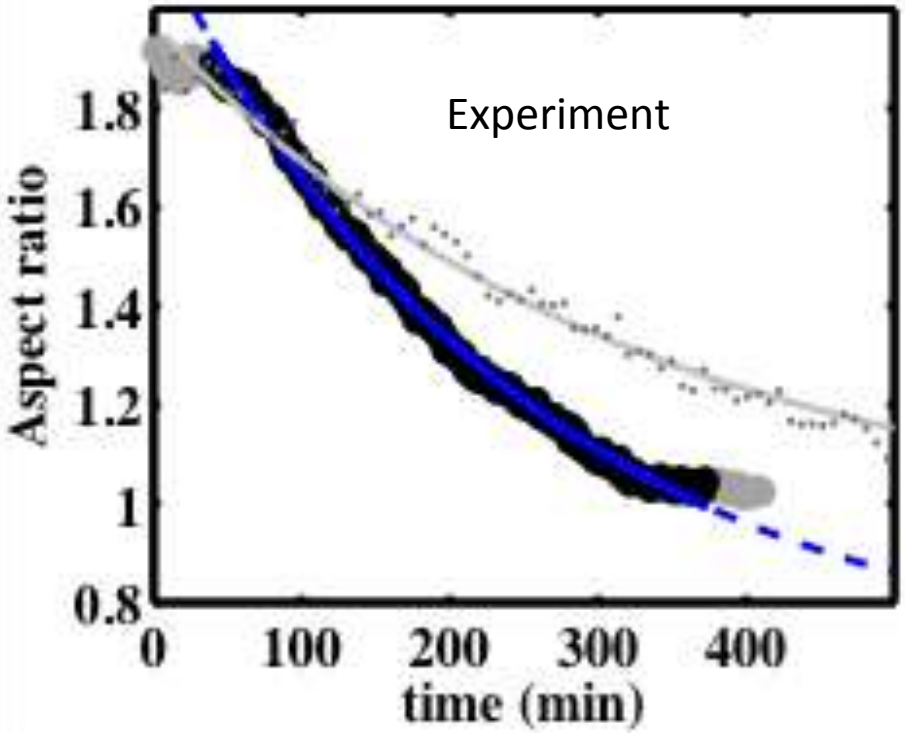


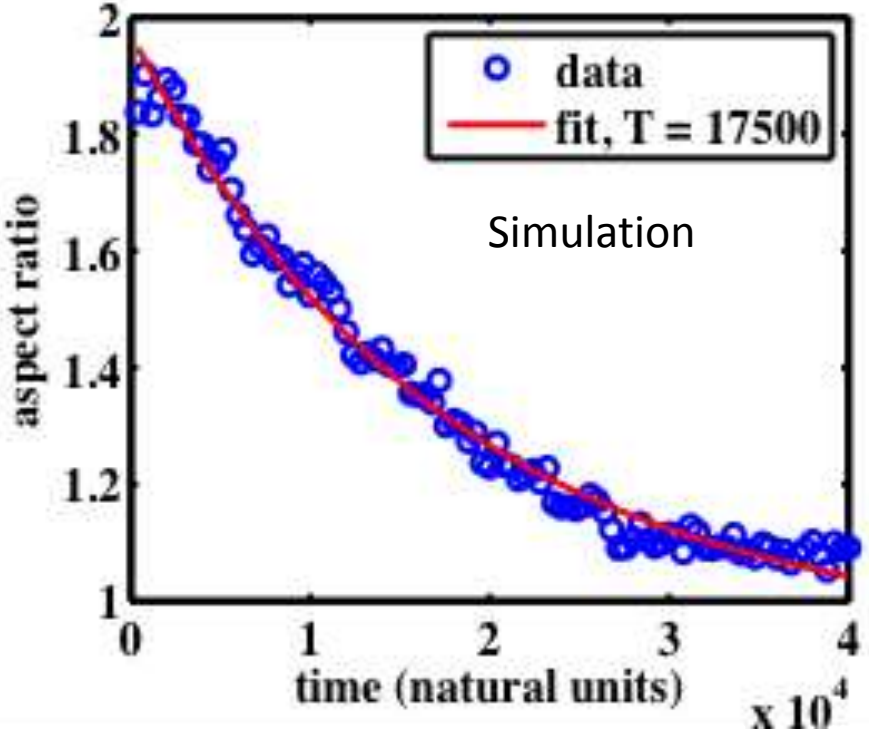


functional form is the same

- Accounting for difference in volume and surface tension (Young, 1939) with no fit parameters
  - relaxation time: experiment = 100 min, simulations = 160 min, GOOD

Fusion assay predictions?





- Qualitative behavior and functional form is the same
- Accounting for difference in volume and surface tension (Young, 1939) with no fit parameters
  - relaxation time: experiment = 100 min, simulations = 160 min, GOOD

Conclusions: Part II

- Tissue “material” properties important in development
- Our simple model accurately predicts bulk visco-elastic properties
  - Only 3 parameters, almost like a supercooled liquid
    - difference: multiplicative “noise” with memory
  - intracellular details not important for bulk behavior
  - qualitatively captures surface behavior
- But, quantitative surface properties in dynamic model are weird
  - actual surface tension much too big
  - this matches long-standing paradox

Summary, I & II

- Part I: cell shapes matter for determining surface properties
  - it also turns out that temporary cell “polarization” could be important, too
- Part II: cell shapes don’t matter too much for bulk properties
  - but we get surface properties wrong because we don’t account for shape changes and polarization
- This suggests that embryonic tissues are a special type of viscoelastic fluid with very different properties from “normal” materials
  - extremely good at forming boundaries
  - cells able to move around easily within those boundaries
  - good for embryonic development?

Thanks for your attention!

Questions?

also, email: mmanning@syr.edu

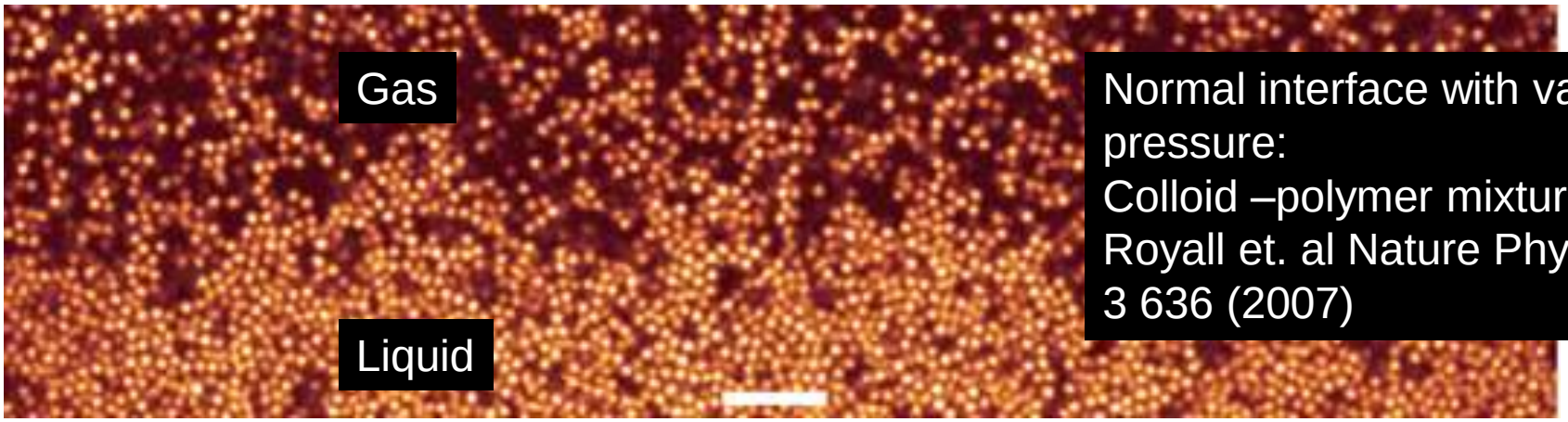
Ellipsoidal to spherical droplet

- Young 1939

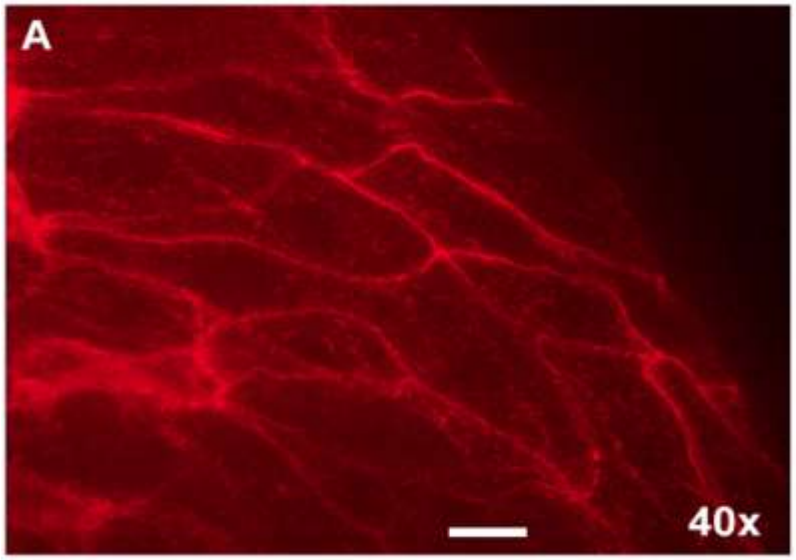
$$r_a = a/b$$

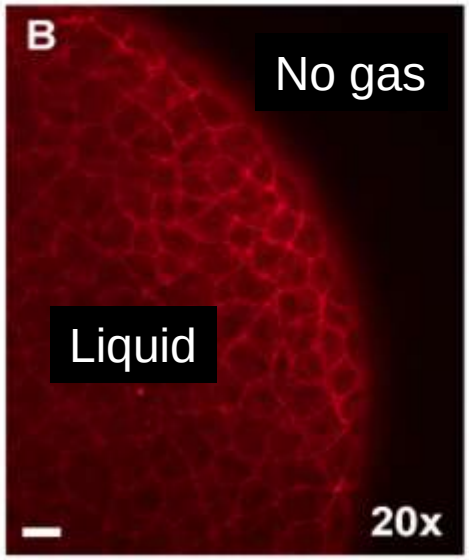
$$\frac{d(r_a)}{dt} = \frac{1}{V^{1/3}} \frac{\sigma}{\eta} f(r_a)$$

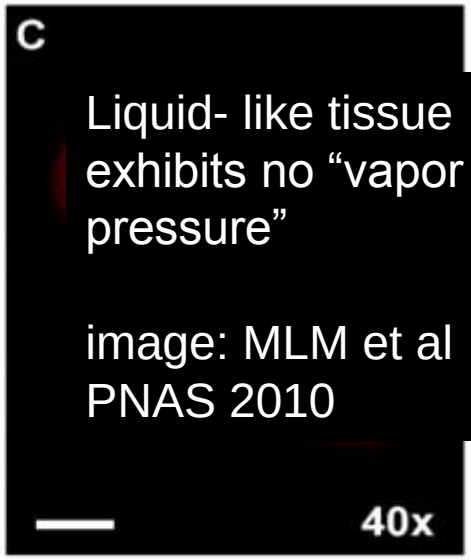
Note: No cell loss is strange!



Normal interface with vapor pressure:
Colloid –polymer mixture
Royall et. al Nature Physics 3 636 (2007)



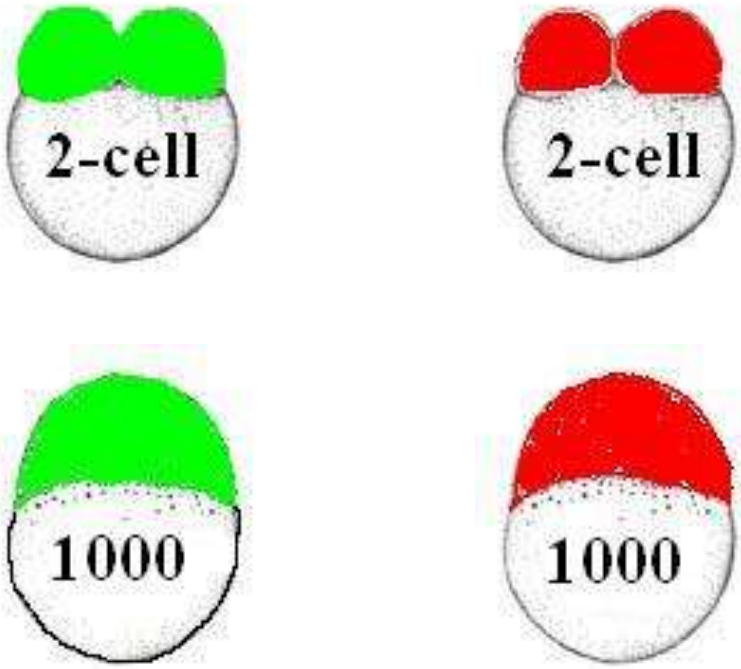




Liquid- like tissue exhibits no “vapor pressure”

image: MLM et al PNAS 2010

Specific tissue generation:



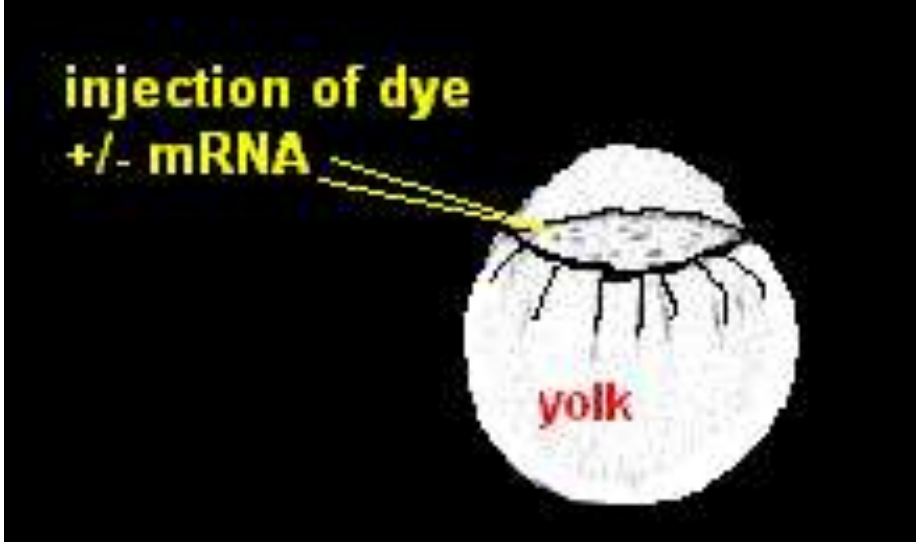
mesendoderm

Cyclops mRNA

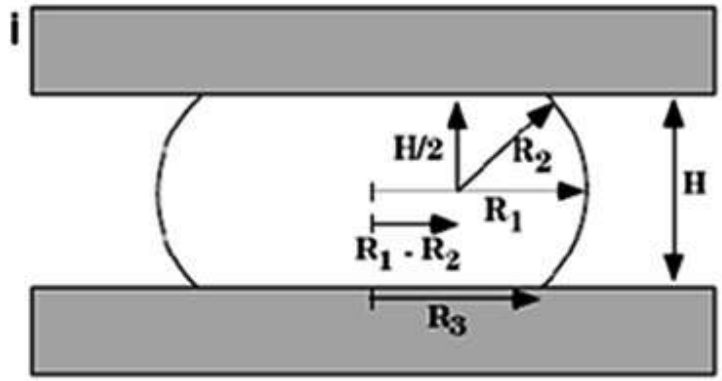
ectoderm

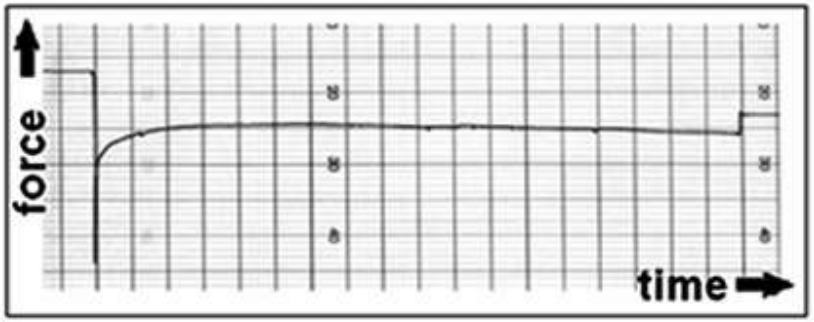
Lefty mRNA

MZ*oep* mutant



Measuring surface tension





$$\frac{F_{eq}}{\pi R_3^2} = \sigma\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$

DMT contact model

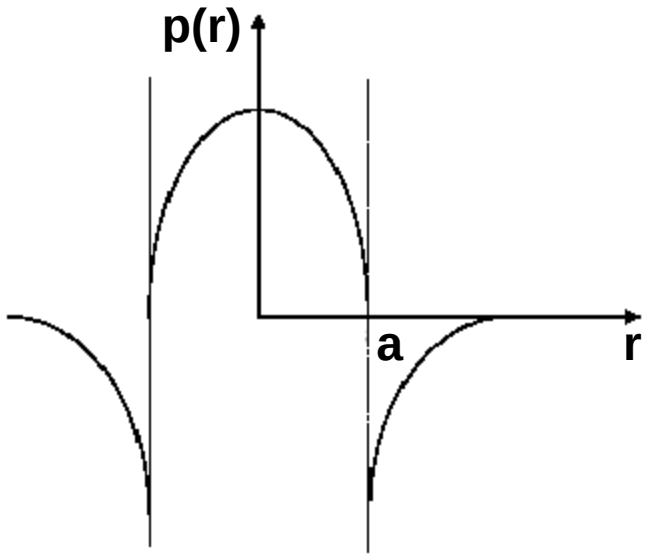
$$0 = \vec{F}_{pot} + \vec{F}_{damp} + \vec{F}_{active}$$

Derjaguin, B.V., Muller, V.M., Toporov, Y.P., 1975, J. Coll. Interf. Sci., 53, pp. 314-326.
Muller, V.M., Derjaguin, B.V., Toporov, Y.P., 1983, Coll. and Surf., 7, pp. 251-259.

- Tensile stresses exist ***outside*** the contact area.
- Stress profile remains Hertzian ***inside*** the contact area.

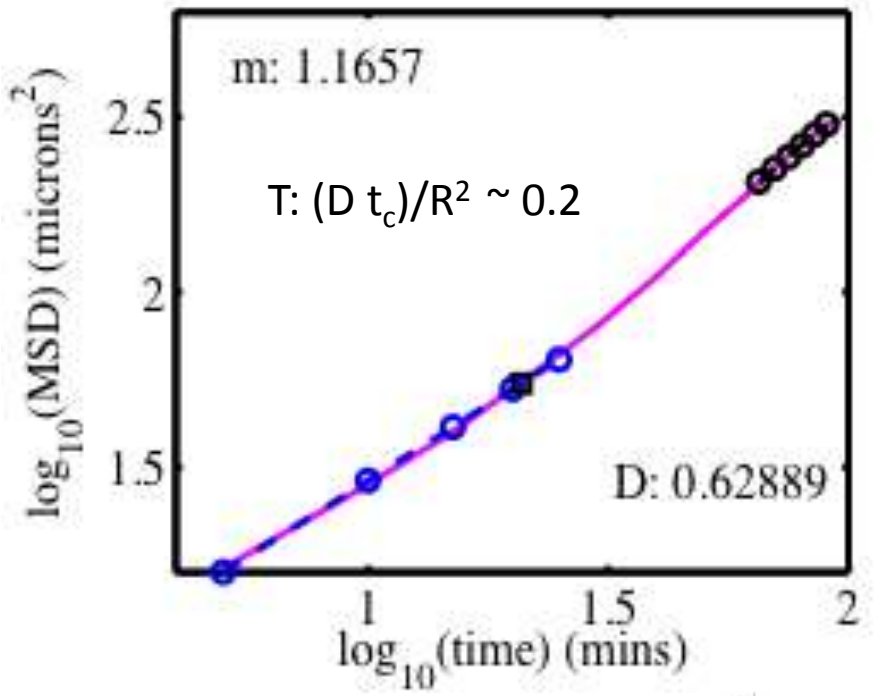
$$\frac{a^3 K}{R} = P + 2\pi\Delta\gamma R, \qquad \delta = \frac{a^2}{R}$$

$$P_{Pull-Off} = 2\pi\Delta\gamma R$$

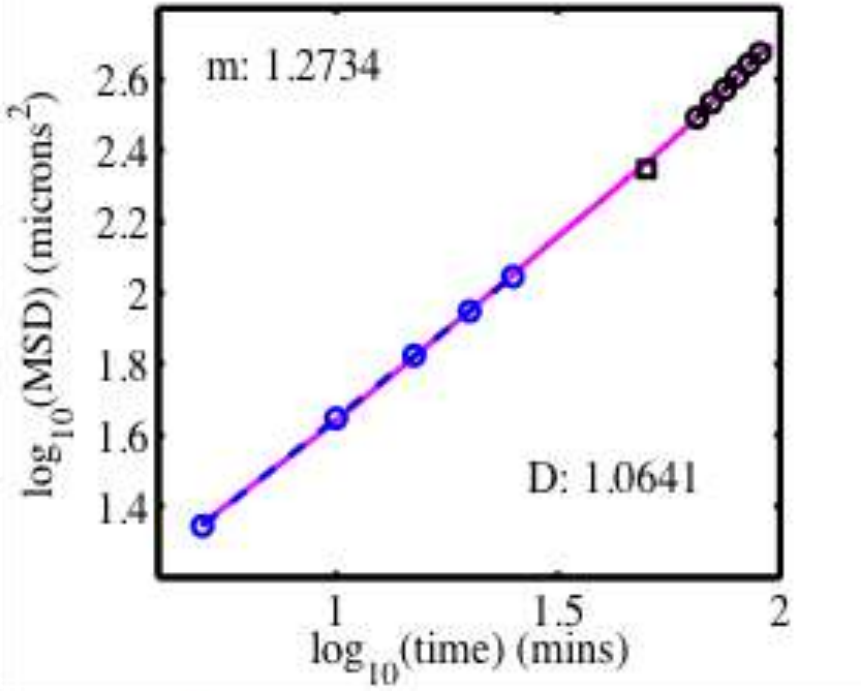


Cell dynamics: nuclei tracking

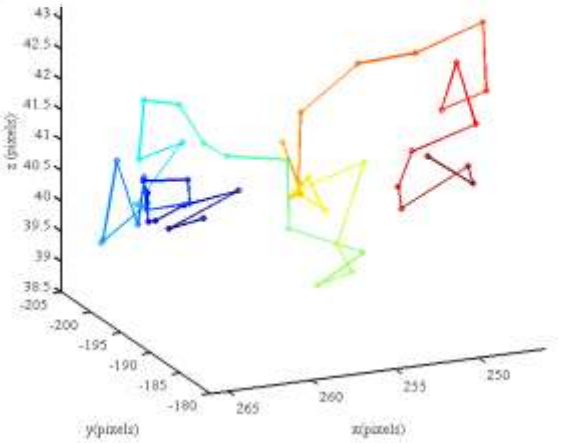
Ectoderm

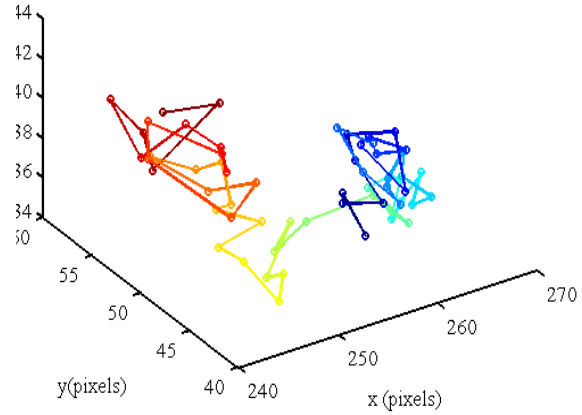


Mesendoderm

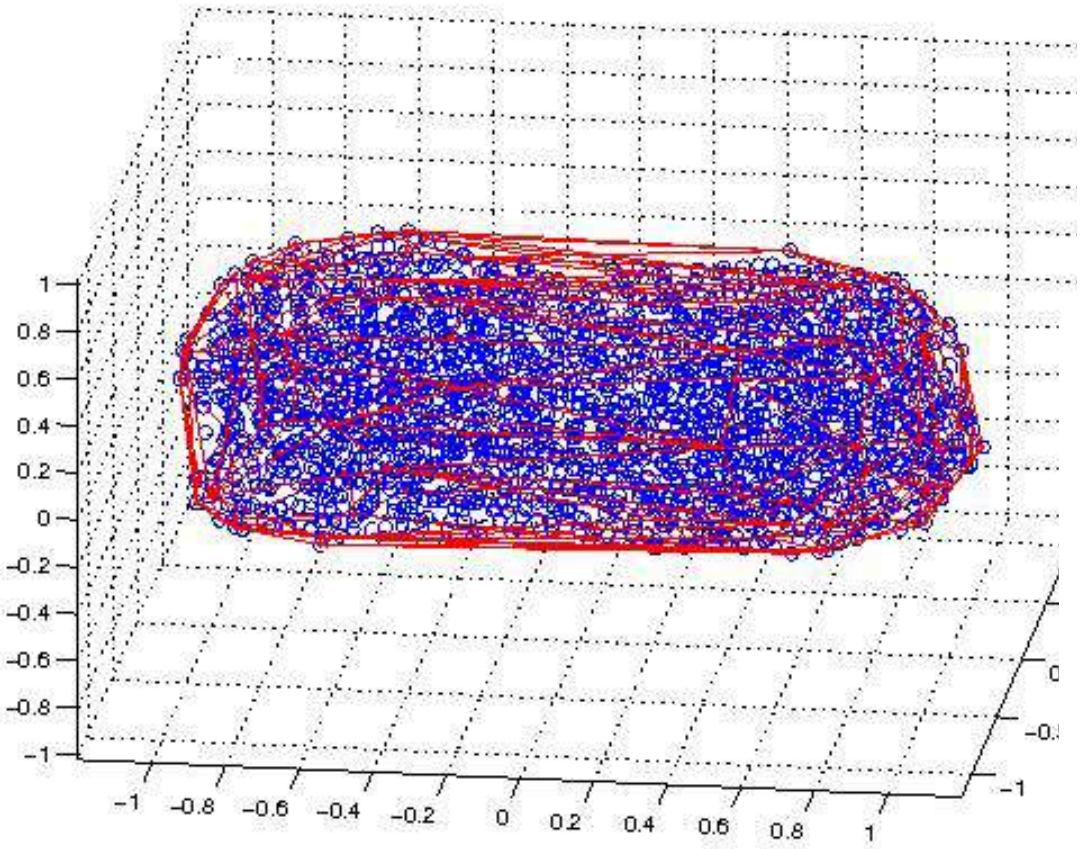


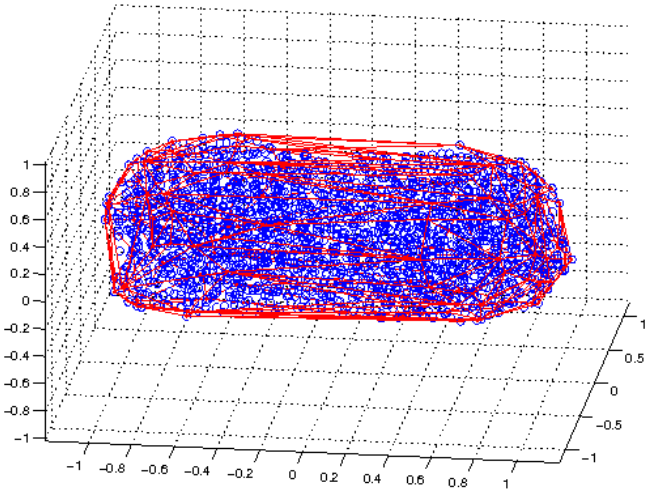
Individual cell tracks and subdiffusive behavior is reminiscent of "supercooled" or "glassy" dynamics

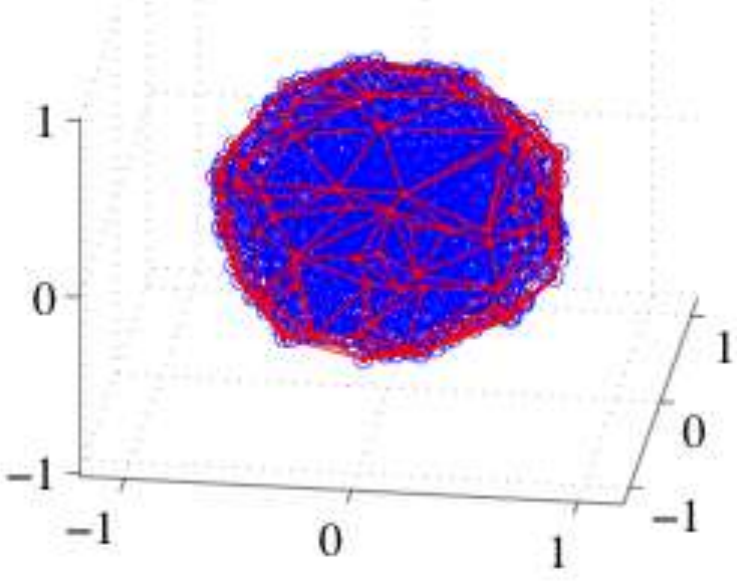




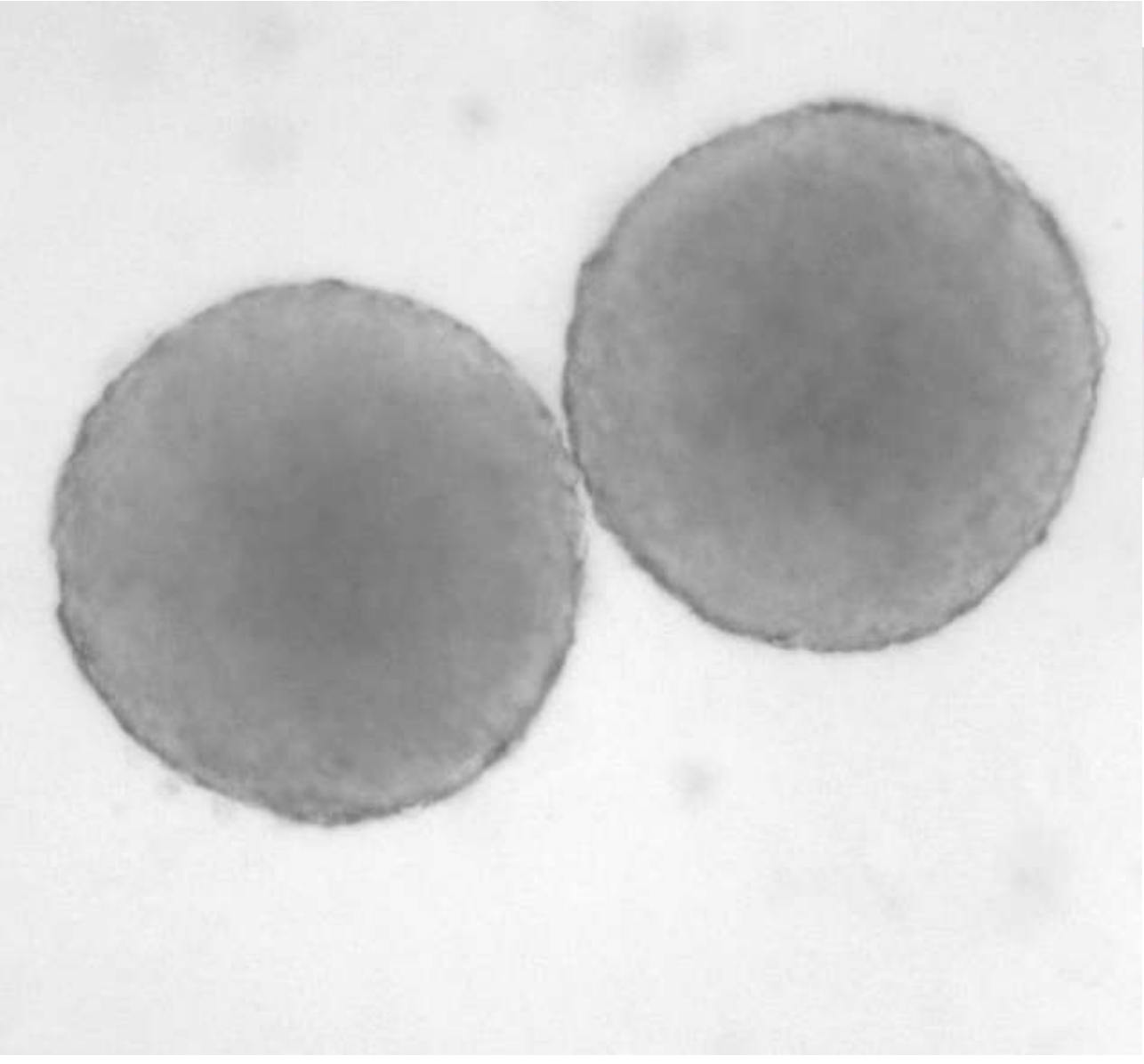
Simulation fusion assay

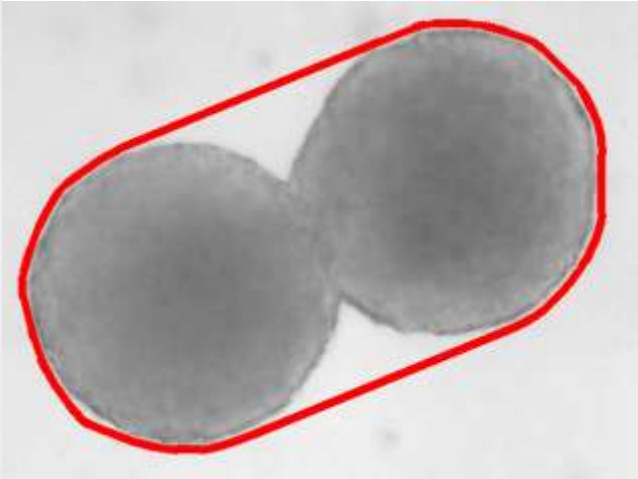


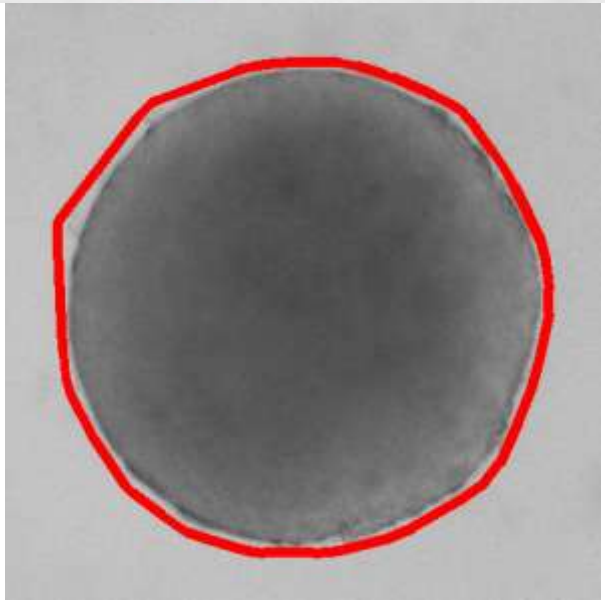


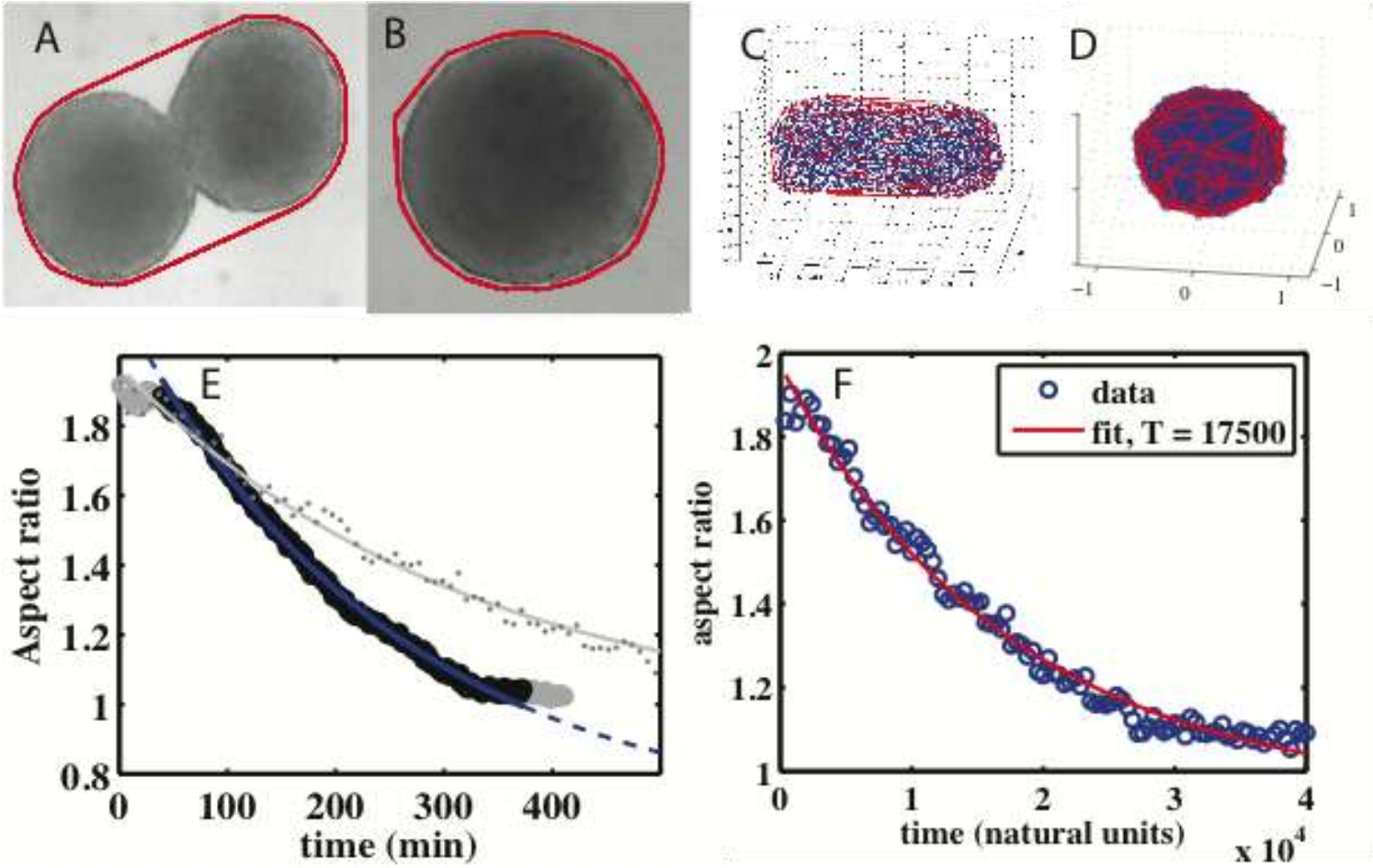


Experimental fusion assay









Calculation for elastic relaxation timescale:

- Actin rheology: Palmer A, Mason TG, Xu J, Kuo SC, Wirtz D (1999) Diffusing wave spectroscopy microrheology of actin filament networks. Biophys J 76:1063–1071.
  - μ for individual cells: 1-10 Pa
  - η for individual cells: 4×10^{-3} Pa s
  - mesh size l for actin network: 100 nm
  - $b = \eta R^3/l$ (lower bound, probably)
  - $\tau = b/(\mu R) \sim$ 1-10 s

Tissue model

- active force conserves momentum, depends on location of neighbors, persists for finite time
  - Natural units: cell radii (R), damping coefficient (b), and effective elastic modulus for single cells (K)
  - elastic relaxation timescale τ = b/(KR)
  - 3 dimensionless parameters (Γ, σ, ξ):

$$\frac{\widetilde{dr_i}}{\widetilde{dt}} = -\sum_j \left[\left(\left(\widetilde{R}_{ij}^{eff} \right)^{1/2} (\widetilde{R}_s - \widetilde{d}_{ij}) - \widetilde{\Gamma} \widetilde{R}_{ij}^{eff} \right) \hat{r}_{ij} + \widetilde{\sigma}\, \xi\, \hat{a}_{ij} \right]$$

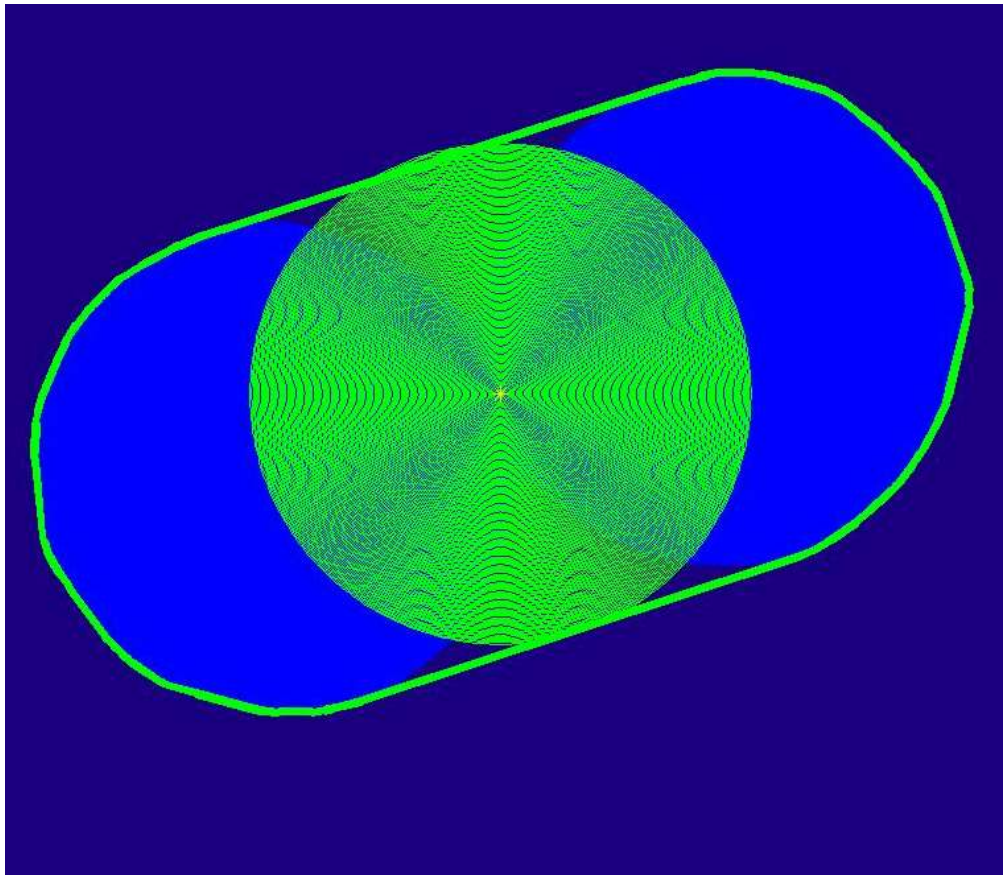
resistance to shape changes (effective modulus)

Γ: Adhesion energy/ Elastic modulus

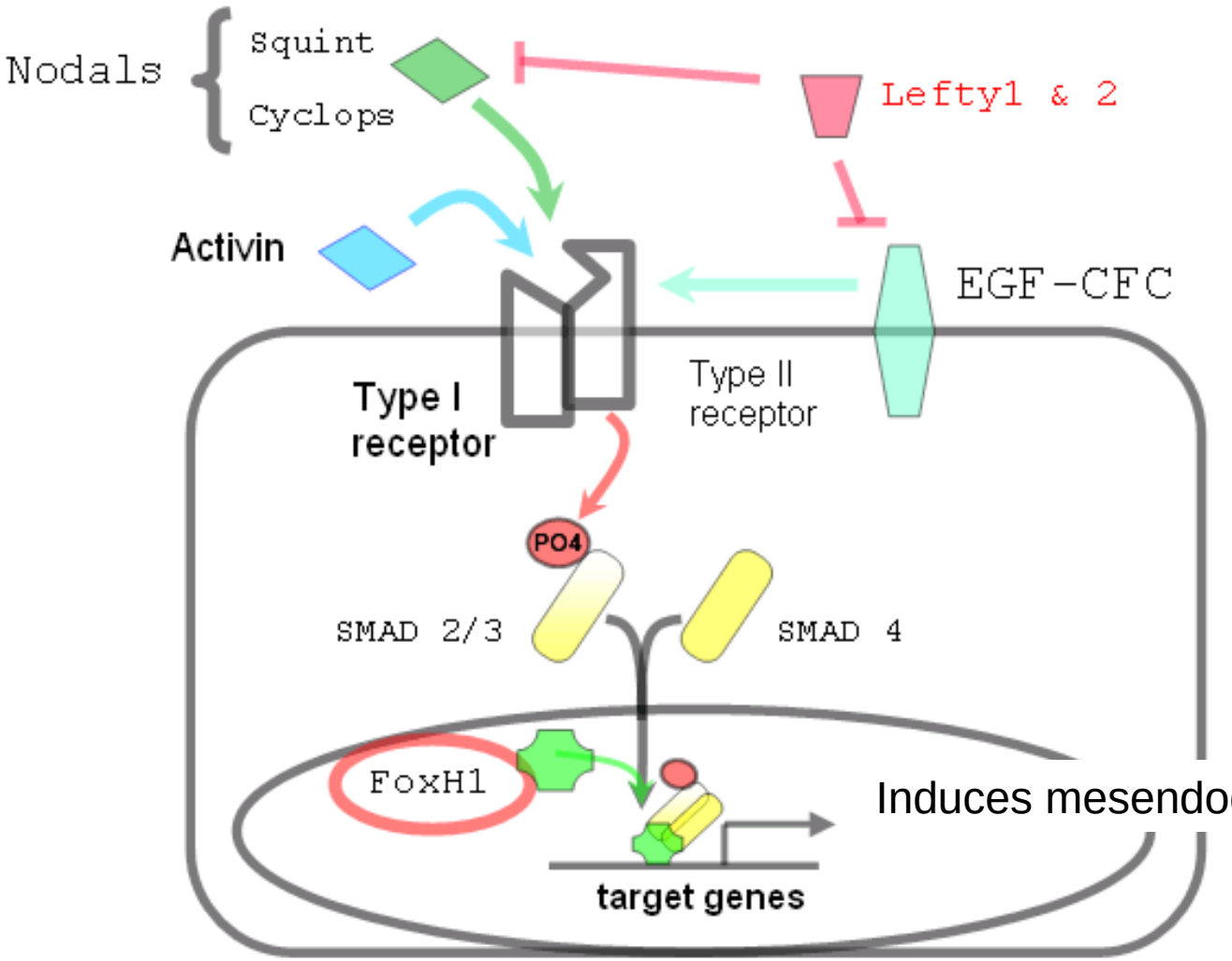
σ: active force magnitude / Elastic modulus

ξ: random variable with persistence time of cell motion/ elastic relaxation

Fusion Assays



Nodal signaling



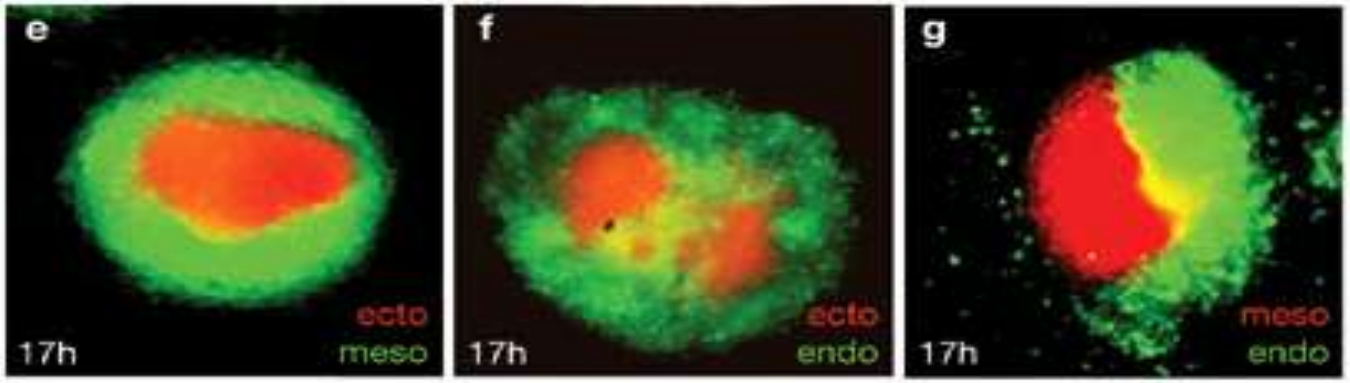
Induces mesendoderm call fate

http://www.genome.gov/Pages/Research/Intramural/nodal_signaling_pathway.htm

Surface tension in different tissues

- Surface tension ordering (highest to lowest)

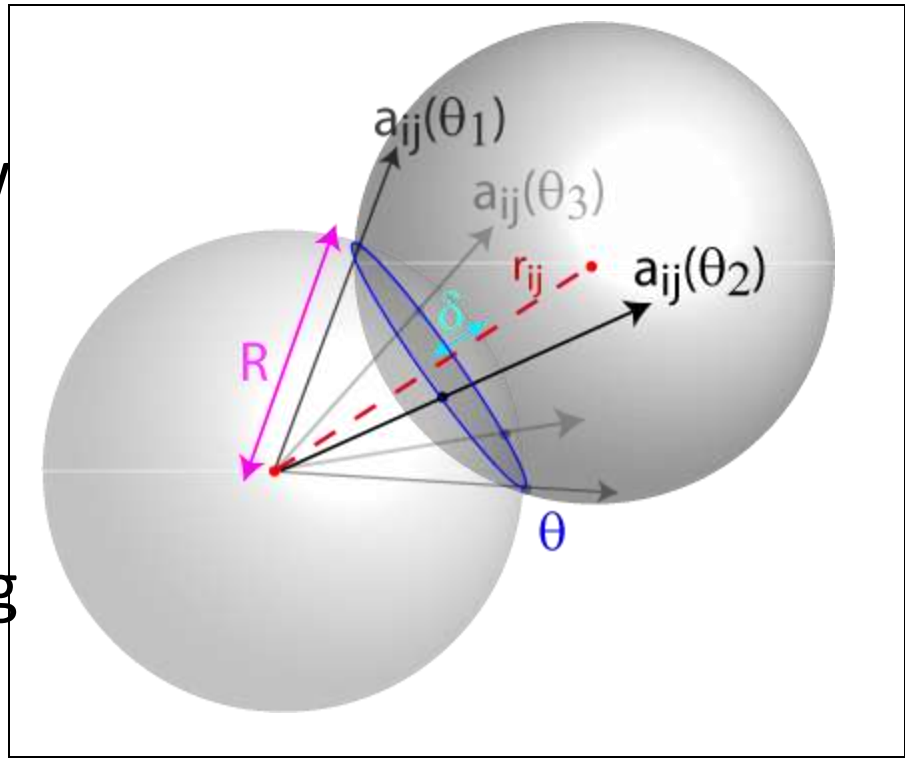
1. Ecto
2. Meso
3. Endo



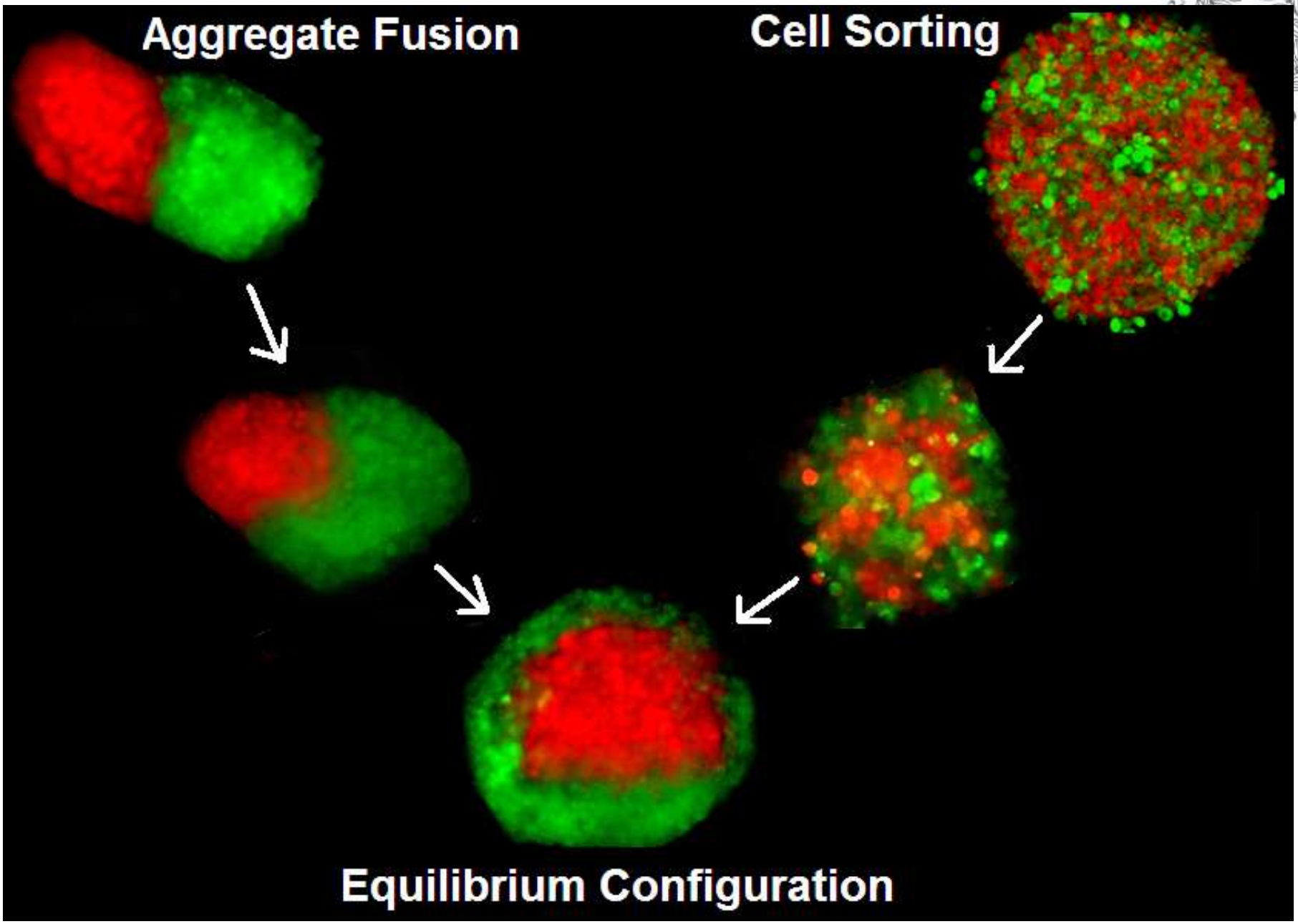
Krieg et al, Nature Cell Biology 10, 429 - 436 (2008)

Active force (continued)

- Protrusions effective in a ring where spheres overlap
- forces obey Newton's 3rd law
- $|F^*|$ is magnitude of force
- η is normally distributed random variable
- a_{ij} is unit vector in direction θ chosen uniformly from ring



Noise depends on the current state $\{ r_{ij}\}$ – multiplicative noise!


Aggregate Fusion
Cell Sorting
Equilibrium Configuration

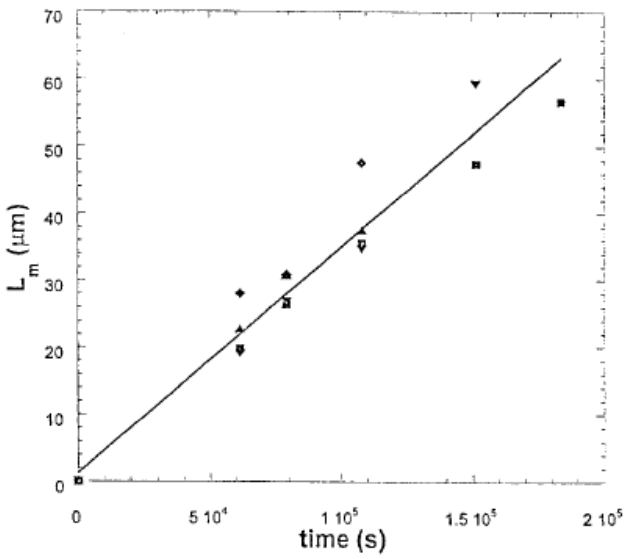
Claim: Surface tension generated by differential adhesion

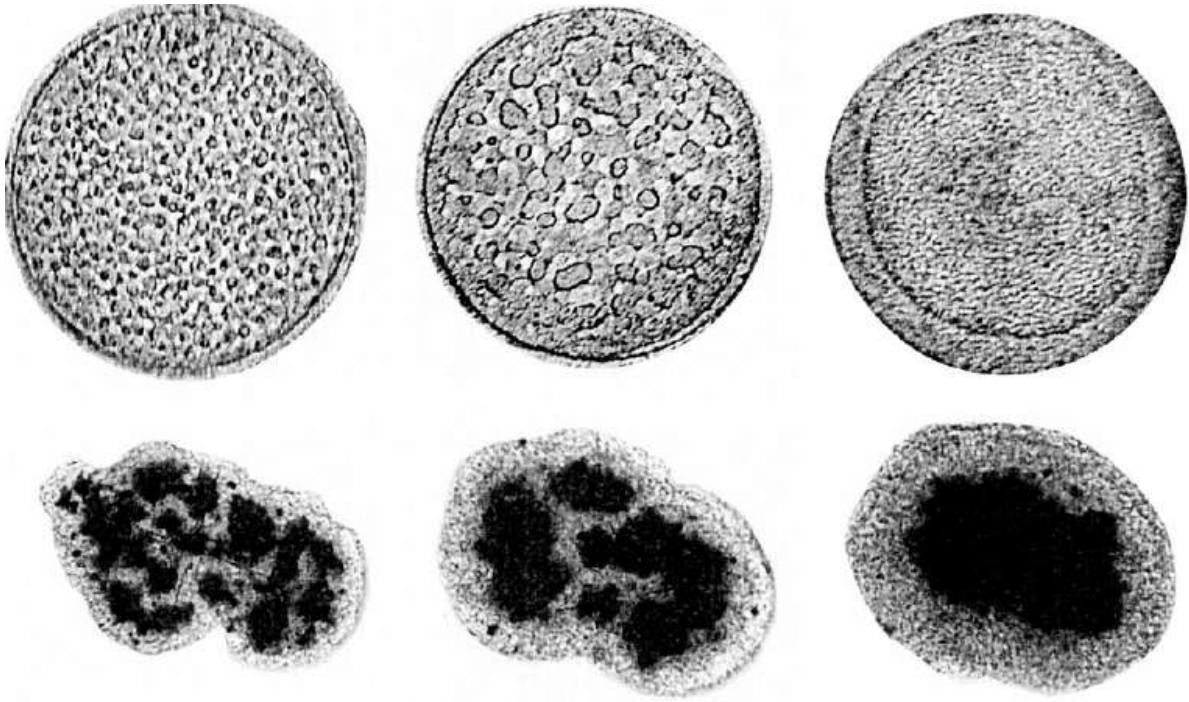
- Just like quenched binary fluids (?)

Cell sorting is analogous to phase ordering in fluids

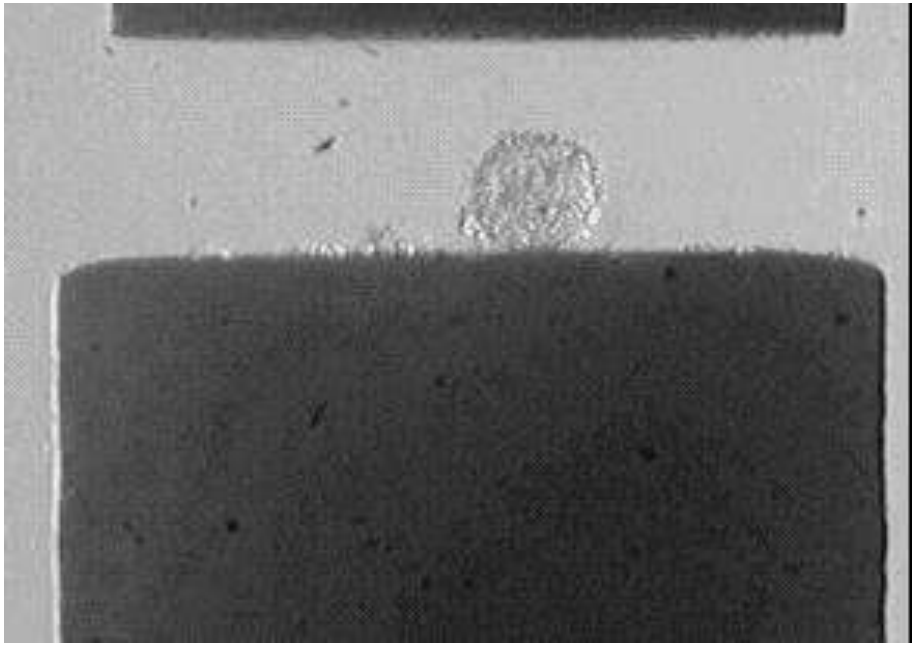
D. A. Beysens*, G. Forgacs†‡, and J. A. Glazier§¶

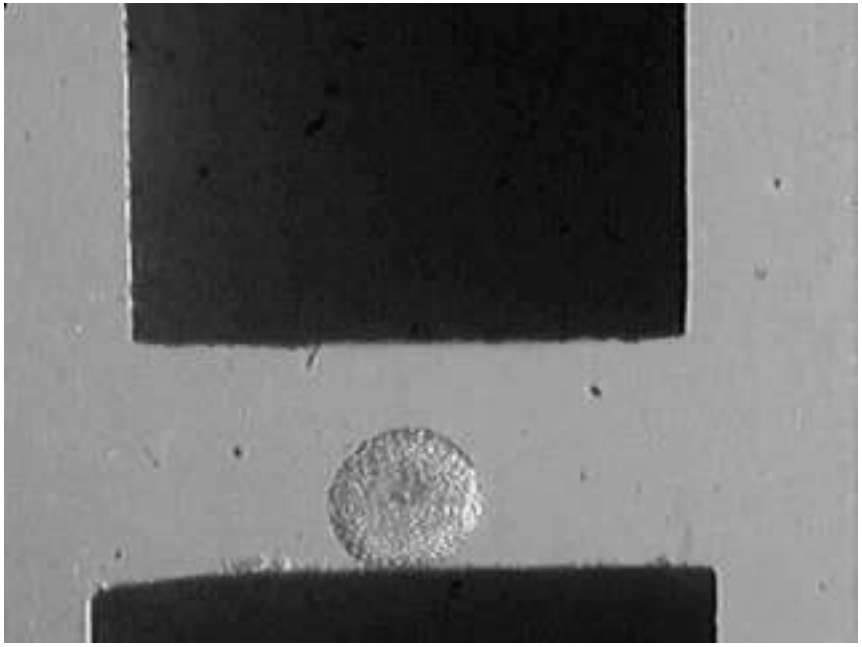
PNAS | August 15, 2000 | vol. 97 | no. 17 | 9467–9471





Different tissue types have characteristic mechanical properties

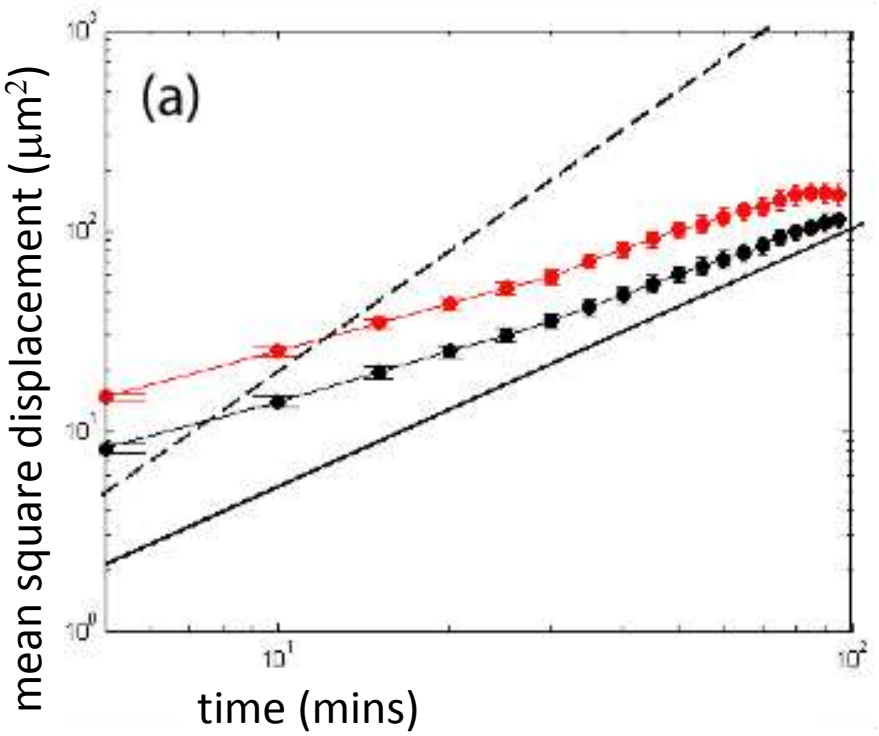


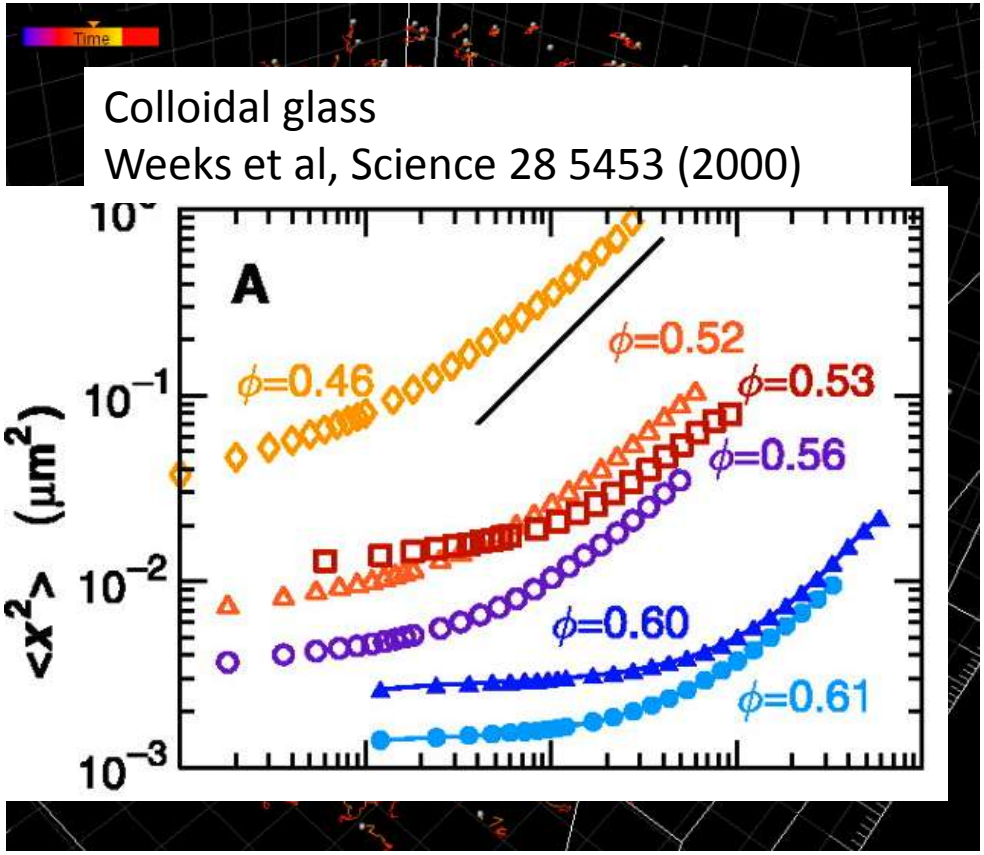


Potential: contact mechanics

- Two types of contact models
  - JKR
    - most realistic
    - hardest to parameterize
    - not proportional to exposed surface area, so not perfect surface tension effect
  - DMT
    - less realistic
    - easy to parameterize
    - proportional to exposed surface area, so acts like a real surface tension
- Both models can be extended to include hysteresis.

Cell trajectories also change with timescale





How is structure related to flow in tightly packed, disordered materials?